

The Journal of Experimental Biology

EDITED BY
J. GRAY

ASSISTED BY
The Hon. Secs. Society for Experimental Biology

Contents

	PAGE
M. M. SWANN. Protoplasmic structure and mitosis. I. The birefringence of the metaphase spindle and asters of the living sea-urchin egg. (With Plate 8 and Nine Text-figures)	417
M. M. SWANN. Protoplasmic structure and mitosis. II. The nature and cause of birefringence changes in the sea-urchin egg at anaphase. (With Plates 9 and 10 and Twelve Text-figures)	434
D. A. PARRY. Factors determining the temperature of terrestrial arthropods in sunlight. (With Eight Text-figures)	445
D. B. CARLISLE. On the hormonal and neural control of the release of gametes in ascidians. (With One Text-figure)	463
MARGARET E. BROWN. The growth of brown trout (<i>Salmo trutta</i> Linn.). IV. The effect of food and temperature on the survival and growth of fry. (With Nine Text-figures)	473
C. ELLENBY. Body size in relation to oxygen consumption and pleopod beat in <i>Ligia oceanica</i> L. (With Seven Text-figures)	492
G. V. T. MATTHEWS. The experimental investigation of navigation in homing pigeons. (With Twelve Text-figures)	508
G. CALCUTT. The role of radiation in photodynamic action. (With Two Text-figures)	537
R. A. BEATTY AND M. FISCHBERG. Cell number in haploid, diploid and polyploid mouse embryos. (With One Text-figure)	541
F. R. HARDEN JONES. The swimbladder and the vertical movements of teleostean fishes. I. Physical factors. (With Three Text-figures)	553
ERIC PONDER. Fragmentation of red cell ghosts in relation to the problem of red cell structure. (With One Text-figure)	567
ANITA M. MANDL. The phases of the oestrous cycle in the adult white rat. (With Two Text-figures)	576
ANITA M. MANDL. Cyclical changes in the vaginal smear of adult ovariectomized rats. (With Four Text-figures)	585
Index	593

Published for The Company of Biologists Limited

CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON

AGENT FOR U.S.A.

ACADEMIC PRESS INC.,

125, EAST 23RD STREET, NEW YORK, U.S.A.

THE JOURNAL OF PHYSIOLOGY

NOVEMBER 1951. VOL. 115, NO. 3

BEKKER, JO M. and KRIJGSMAN, B. J. Physiological investigations into the heart function of *Daphnia*.

DAWES, G. S., MOTT, J. C. and WIDDICOMBE, J. G. Respiratory and cardiovascular reflexes from the heart and lungs.

TASKER, RONALD R. The collection of intestinal lymph from normally active rats.

LADELL, W. S. S. Assessment of group acclimatization to heat and humidity.

ROBSON, J. M. and SHARAF, A. A. Response of the cat's endometrium to implantation of different steroids.

WINTER, L. B. Skin tests on serum-sensitized guinea-pigs.

FATT, P. and KATZ, B. An analysis of the end-plate potential recorded with an intra-cellular electrode.

16s. 6d. net

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1

PROTOPLASMIC STRUCTURE AND MITOSIS

I. THE BIREFRINGENCE OF THE METAPHASE SPINDLE
AND ASTERS OF THE LIVING SEA-URCHIN EGG

By M. M. SWANN

*From the Department of Zoology, University of Cambridge,
and the Marine Station, Millport*

(Received 15 March 1951)

(With Plate 8 and Nine Text-figures)

INTRODUCTION

The mitotic figures of many different living cells are now known to be birefringent. The first observation of this kind was made by Runnström (1928) on the eggs of the sea-urchin *Psammechinus miliaris*. Since then birefringence has been described for a number of mitotic figures: in the eggs of the same sea-urchin by Schmidt (1937, 1939) and Monné (1944); in the eggs of *Chaetopterus* by Inoué (1950); in the cells of the chick by Hughes & Swann (1948), and in the cells of *Tradescantia* by Kuwada & Nakamura (1934). I myself have seen birefringence in the mitotic figures of amphibian, bird and mammal tissue cultures; in the eggs of nematodes, annelids, molluscs and echinoderms, and in the spermatocytes of Orthoptera. Since this birefringence is so widespread, and since spindles and asters are, by definition, oriented structures, it is likely that all mitotic figures are birefringent. However, this may not always be apparent in living cells, because of the opposing effects of form and intrinsic birefringence (Swann & Mitchison, 1951).

The only detailed study of the birefringence of mitotic figures is that of Schmidt (1937, 1939). He found that the spindle of *Psammechinus miliaris* was most strongly birefringent at metaphase, being positive with respect to its long axes. Since protein fibres are also positive with respect to their long axis, he supposed the spindle to consist of fibres arranged lengthwise. The asters show positive radial birefringence, and he supposed that they consist of fibres arranged radially. He also found that the birefringence of the whole mitotic figure disappeared gradually in anaphase, and attributed this to a contraction of the fibres, since the birefringence of structures such as muscle also decrease on contraction. Though not conclusive, these observations have lent considerable weight to the traction fibre hypothesis of mitosis.

Many hypotheses have been put forward at various times to account for chromosome separation (Schrader, 1944). Most of them, however, are quite inadequate to account for the process, and in a valuable review, Cornman (1944) comes to the conclusion that only a traction fibre hypothesis is free from serious objection. A study by Hughes & Swann (1948) on the form of anaphase movement curves led to the same conclusion. On the other hand, there is evidence in many cells that the spindle elongates during anaphase, and that this elongation is partially, and in a few

cells even largely, responsible for chromosome separation (Ris, 1943). Traction and elongation, however, are not mutually exclusive, and as Ris has pointed out, it is possible to classify mitoses according to the relative contributions of the two mechanisms to the total chromosome movement.

Unlike some hypotheses of mitosis, which have supposed the spindle to be no more than a passive guide for chromosomes which are repelling each other, traction and elongation both imply an active function. It seemed important, therefore, to know something more of the changes that take place in the spindle, and it is for this reason that I have attempted a quantitative study of birefringence throughout the mitotic cycle. The results of this study will be presented in a number of separate papers.

In this, the first paper of the series, the structure of the spindle and asters at metaphase is analysed in a preliminary way. In the second paper (Swann, 1951*a*), the changes during anaphase are examined. These two papers will show that various structural changes take place in the spindle and asters during anaphase, probably caused by the release of certain substances from the chromosomes. Later papers will deal with structural changes in the cortex and cytoplasm at other points in the mitotic cycle, and with questions of form and intrinsic birefringence. From this evidence, a hypothesis of mitosis is developed (Swann, 1951*b*, 1952), based on the idea that structural agents, concerned with altering protoplasmic patterns, periodically condense on to, and diffuse away from, the chromosomes. In some joint work with J. M. Mitchison, these ideas are extended to the cleavage process (Mitchison, 1952).

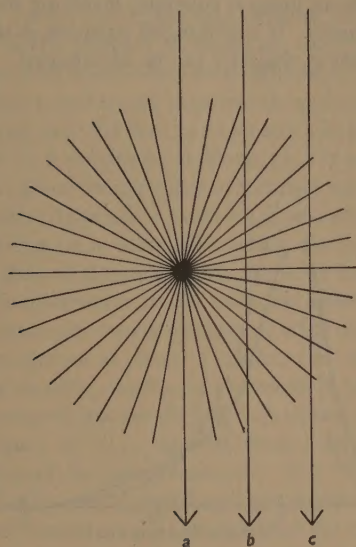
MATERIAL AND METHODS

Most of the work described in these papers was done at the Marine Station, Millport, using the eggs of the *Psammechinus miliaris*. The metaphase spindle in these eggs has a maximum retardation* of about $\lambda/100$, or 50A, so that it is easily detectable with a polarizing microscope of ordinary sensitivity. In the early and late stages of mitosis, however, the retardations may be as low as 1 or 2A., and increased sensitivity becomes essential. The means for obtaining this increased sensitivity have been described elsewhere (Swann & Mitchison, 1950). The sea-urchin egg, however, presents a particular difficulty, in that its numerous granules scatter a large amount of light. At objective N.A.'s of 0.65 or more, this scattered light entirely obscures the weaker retardations. All the observations were therefore made with a $10\times$ objective of N.A. 0.28. Under these conditions the scattered light is greatly reduced.

Most of the photographs were taken with a 16 mm. time-lapse camera. Using polarized light, the level of illumination at the image plane is very low, so that the fastest emulsions, such as Kodak Super XX or Kodak R 55 are essential. For the same reason a carbon arc was used as the light source, with a Chance ON 19 heat filter. The magnification on the film was usually about $100\times$, a compensator was used, and the condenser N.A. was standardized at 0.12. Under these conditions the exposure required varied from 5 to 15 sec. Time-lapse rates varied between 2 and 6 frames per min. For still photography a 35 mm. camera was used.

* These and other terms in polarized light microscopy are briefly explained in Appendix 1.

The retardation at any point in the spindle and asters was calculated from the density of the film negative. To do this, a densitometer was built to work on patches of film of about 100μ square. This densitometer, and the methods of calculating retardations from density measurements, have been described by Swann & Mitchison (1950). The light scattered by the granules in the sea-urchin egg, however, raises certain special problems in the calculation of retardation which are discussed in Appendix 2.



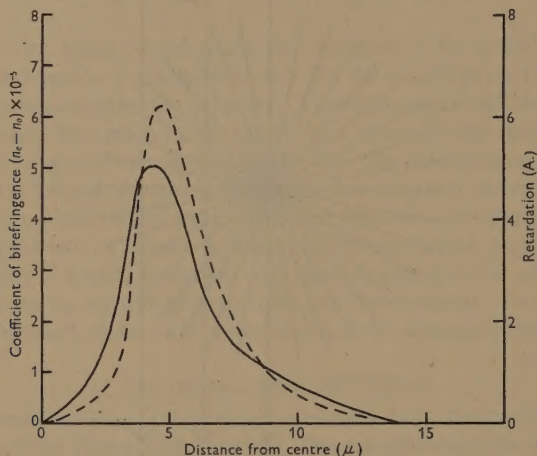
Text-fig. 1. Diagram to illustrate the build-up of retardation in a radially symmetrical structure.

THE ANALYSIS OF ASTER BIREFRINGENCE

The structure of the aster has been the source of continual speculation. On one point only is there agreement: that it is radially symmetrical body. Whether it consists of discrete fibres radiating from a centre, or whether it is a homogeneous body with a radial structure, is uncertain. The simple fact that it is radially symmetrical, however, makes it possible to determine the coefficient of birefringence ($n_e - n_o$) at any point within it. As will become apparent, this information goes some way to settling the controversies about its structure.

The retardation of an aster, illuminated by a thin pencil of polarized light, is dependent on the birefringent elements traversed by the pencil. Pencil *a* in Text-fig. 1, for instance, traverses elements from the centre to the periphery of the aster, but always parallel to their long axes. Pencil *b* traverses elements near the centre more or less normally, and elements further out at an angle. Similarly for pencil *c*. Bearing in mind that the coefficient of birefringence for a unit thickness of astral

material is the same at a given distance from the centre along any radius (because of the radial symmetry of the aster), it would be possible, if the variation in coefficient of birefringence with distance were known, to calculate by summation the retardation to be expected in any pencil *a*, *b*, *c*, etc., having regard to the angle at which the various elements were traversed. In fact the position is reversed. The retardations in pencils *a*, *b*, *c*, etc., are known, but not the variation in coefficient of birefringence with distance from the centre. Mathematically, however, the retardation of the aster can be expressed as an integral equation, involving the coefficient of birefringence of the astral elements. If this integral equation is solved, the coefficient of birefringence at any point in the aster can be determined.



Text-fig. 2. Retardation (continuous line) and coefficient of birefringence (dashed line) of a metaphase aster. These curves are of the lower aster of Pl. 8, fig. 1.

By good fortune, the integral equation can be solved exactly.* From a curve of aster retardation against distance from the centre, it is possible therefore, by applying the appropriate procedures, to construct a curve of the coefficient of birefringence against distance from the centre. The derivation of the integral equation and the solution are given in Appendix 3.

Metaphase mitotic figures of one- and two-cell stages are shown in Pl. 8, figs. 1-3. The asters are evident in each case, at either end of the strongly birefringent spindle. The centres are isotropic, but at a short distance out, the retardation rises to a maximum, and then falls gradually to nil. The continuous curve of Text-fig. 2 shows this variation of retardation with distance quantitatively. The dashed curve shows the coefficient of birefringence, derived from the retardation curve.

The close similarity between the curves of retardation and coefficient of birefringence is surprising at first sight. This similarity comes about because most of the retardation of an aster is produced by material more or less normal to the line of

* I am indebted to Mr Freeman Dyson for solving this equation.

vision. The retardation due to elements at smaller angles to the line of vision is only slight, since they are for the most part at some distance from the centre, where there is a low coefficient of birefringence, while the fact that they are seen at a small angle still further reduces their contribution to the total retardation. As a result, the curve for retardation is, as regards its shape, a fair approximation to the curve for coefficient of birefringence.

In many types of cell, the asters and spindle are generated by sharply defined granules, the centrioles. In the sea-urchin egg, however, there is no distinct granule, but a diffuse body of a few microns in diameter, the centrosome. It might be expected that the aster curves would show no coefficient of birefringence for a short distance out from the centre, but would then rise abruptly to a maximum value. In fact, there is a gradual rise over the first 2μ , a steeper rise between about 2 and 4μ , and then a gradual rise to a maximum at about 5μ . It is difficult to know what reliance to place on this part of the curve, since there are several effects in the optical system, discussed in detail in Appendix 4, that tend to smooth out sharp discontinuities in the object. Nevertheless, it is doubtful whether they could produce sufficient smoothing to convert a sharp discontinuity into the curve that is actually found. It is possible, therefore, that orientation is not built up suddenly at the edge of the centrosome, but more gradually over a distance of a few microns.

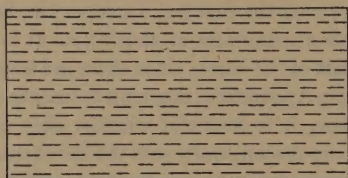
Having reached a maximum at about 5μ from the centre, the coefficient of birefringence falls away steadily, reaching a minimum at about 15μ . Before going on to consider the meaning of this steady fall, it is a matter of some importance to decide whether the figure of 15μ , derived from birefringence measurements, represents the real limits of the metaphase aster. The fibrillar appearance of the aster in living and fixed cells, seen by ordinary light, extends only as far out as this. It is significant too, that Chambers (1924) finds that the metaphase mitotic figure is a relatively small gelated body which can be pushed round the fluid cytoplasm, while Heilbrunn (1943) finds that the bulk of the cytoplasm at metaphase has a low viscosity. The evidence, such as it is, suggests therefore that the limits of the aster as determined from birefringence measurements, do correspond to the limits of oriented structure.

The coefficient of birefringence of the aster falls from about 6×10^{-5} at 5μ from the centre, to a much lower value at the edge. It is not possible to say exactly how low this value is, since it can only be calculated from the very low retardation at the periphery of the aster, and this cannot be measured with much accuracy. There is, however, at least a twenty-fold fall.

For any given material, presumably protein in the case of the aster, the coefficient of birefringence depends on a number of factors:

- (1) Amount of material present.
- (2) Proportion of the total material actually oriented.
- (3) Degree of molecular orientation.
- (4) Degree of micellar orientation.
- (5) Micellar volume (proportion of material organized into micelles).

In simple oriented high polymer systems, the amount of material present does not vary from point to point, and the whole of this material is oriented to a greater or lesser degree (Text-fig. 3 *a*). There is no *a priori* reason, however, for supposing that the amount of material in different regions of the spindle or asters is the same. Nor is it certain that the oriented structure is homogeneous. Oriented fibrils lying in an unoriented matrix, for instance, might give a proportion of oriented material that varied from point to point (Text-fig. 3 *b*). In some birefringent systems, particularly the strongly fibrous and rather inert proteins, it is possible to separate, by means of imbibition experiments, the relative contributions of form and intrinsic birefringence. Assuming that the amount of material present and the proportion of it that is

*a**b*

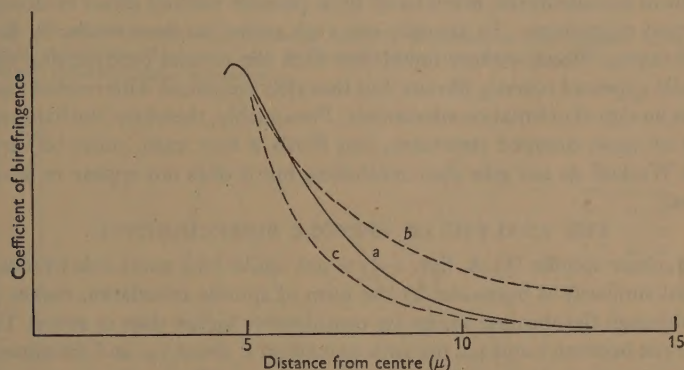
Text-fig. 3. Two types of structure built up from anisodiametric particles. *a*, amount of material and proportion of material oriented constant throughout; *b*, amount of material constant throughout, but only about 50 % of material oriented (fibrils lying in an unoriented matrix). The particles shown are purely diagrammatic, and represent either molecules or micelles.

oriented, remain constant, form birefringence then gives a measure of the degree of micellar orientation and the proportion of material organized into micelles (micellar volume), while intrinsic birefringence gives a measure of the degree of molecular orientation. In the case of the mitotic figures of the sea-urchin egg, however, the protoplasmic structure is labile, and the effect of fixatives and imbibition agents is drastic, so that it is not easy to carry out satisfactory imbibition experiments. A detailed interpretation of molecular and micellar arrangement is therefore difficult, and the problem is only examined in a preliminary way in the present paper. Questions of form and intrinsic birefringence will be considered at length in a later communication.

The amount of material in different regions of the aster is not easily determined. Living asters, examined in ordinary light, show a somewhat reduced refractive index

at their centre, as do unstained sections in phase contrast (the well-known dumb-bell of metaphase) but this seems to be because the centres are free of granules. These granules are mostly basophilic, and readily seen by staining with basic dyes, either vitally or in sections. If, on the other hand, the ground cytoplasm is stained with acid dyes, it takes up the stain uniformly, and there is no sign of variation in protein density from point to point. It should be added that an examination in polarized light, using a suitably high aperture, leaves no doubt that the birefringence of the aster is caused by ground cytoplasm and not by granules. While, therefore, there may be slight differences in protein density, they are quite insufficient to cause the twenty-fold decrease in coefficient of birefringence between the peak and the edge of the aster.

The possibility that the fall in coefficient of birefringence is due to a decrease in the proportion of oriented material is particularly interesting, since one of the classical conceptions of aster structure, of fibres radiating from the centre like the spokes of a wheel, requires for simple geometrical reasons, that the proportion of oriented material should fall on an inverse square law. The coefficient of birefringence of a metaphase aster is therefore plotted beside inverse square and inverse fourth-power curves in Text-fig. 4.



Text-fig. 4. Coefficient of birefringence of a metaphase aster (*a*), compared with an inverse square curve (*b*) and an inverse fourth power curve (*c*). The metaphase aster curve is the same as in Text-fig. 2.

The coefficient of birefringence of the aster reaches a maximum value of 6×10^{-5} , at 5μ from the centre. If the molecular and micellar structure of the aster remained constant throughout, and if the proportion of oriented material did not vary, the coefficient of birefringence would of course remain constant at 6×10^{-5} out to the periphery. If, on the other hand, the proportion of oriented material fell off as an inverse square, as the result of a radiating fibrillar structure, the coefficient of birefringence should fall to about 1.2×10^{-5} at a distance of 12μ . In fact, the coefficient of birefringence falls to about 0.3×10^{-5} at this distance. If therefore the aster is a homogeneous body, with no fall in the proportion of oriented material,

the twenty-fold fall in coefficient of birefringence between 5 and 12μ , has to be accounted for solely in terms of molecular and micellar changes. If, on the other hand, the proportion of oriented material falls on an inverse square, there is only a four-fold drop to be accounted for in these terms.

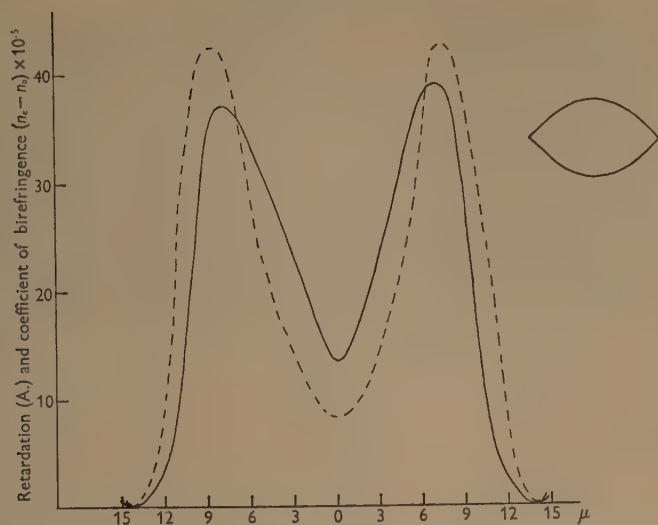
Although there is no reason why molecular and micellar changes should not combine to give a twenty-fold decrease in coefficient of birefringence over a distance of 7μ , there is no obvious mechanism to account for such an effect. A radiating fibrillar structure, on the other hand, provides a simple explanation for the greater part of the fall in coefficient birefringence. It is apparent from Text-fig. 4 that the aster curve is not in fact an inverse square, since it falls away rather too rapidly, but if the fibrils in question were of uneven length, it might be possible to account even for this. Moreover, there are other reasons for thinking that a protoplasmic structure such as the asters and spindle might consist of fibrils, rather than being homogeneously oriented. Muscle, flagella and most oriented protoplasmic structures that have been examined in the electron microscope, have proved to contain discrete fibrils, often of great length. A further suggestive point is that in spermatocytes and in various protozoa, the same centres generate both the spindle and the flagella.

A decision as to whether the mitotic figure is homogeneously oriented, or whether it consists of definite fibrils, is not likely to be possible without direct evidence from the electron microscope. So far only one such study has been made, by Rozsa & Wyckoff (1950). These authors found that with the normal precipitating fixatives the spindle appeared coarsely fibrous, but that after treatment with neutral formalin, there was no sign of orientation whatsoever. Presumably, therefore, the living spindle contains no gross oriented structures, and fibrils if they exist, must be very fine. Rozsa & Wyckoff do not give their resolution, but it does not appear to have been very great.

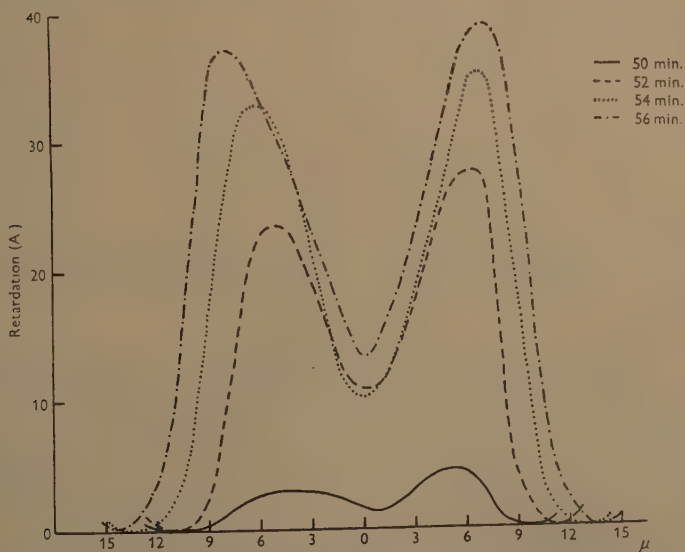
THE ANALYSIS OF SPINDLE BIREFRINGENCE

The metaphase spindle (Pl. 8, figs. 1-3) is not unlike two asters side by side. This superficial similarity is borne out by the form of spindle retardation curves (Text-fig. 5), although the absolute values are considerably higher than in asters. There is the same rise between 2 and 4μ , the same maximum at about 5μ , and the same steady fall with increasing distance from the centrosomes. The similarity is somewhat deceptive however, as can be seen from the retardation curves for different stages in the early growth of the spindle (Text-fig. 6). Even at 50 min., when the spindle has only just become visible, there is appreciable birefringence at the equator. In fact, the equatorial birefringence is relatively as great at this stage as at any other, and there is no suggestion that the spindle arises from the fusion of two asters. This is not altogether surprising, since it is well known that the oriented structure of the spindle is generated not only by the centrosomes or centrioles, but also by the centromeres of the chromosomes. In many spindles there are in fact no centrosomes or centrioles.

The spindle, unlike the aster, is not radially symmetrical, so that it is not possible to formulate and solve an integral equation to calculate coefficient of birefringence. By assuming a definite shape, however, it is possible to calculate approximately the



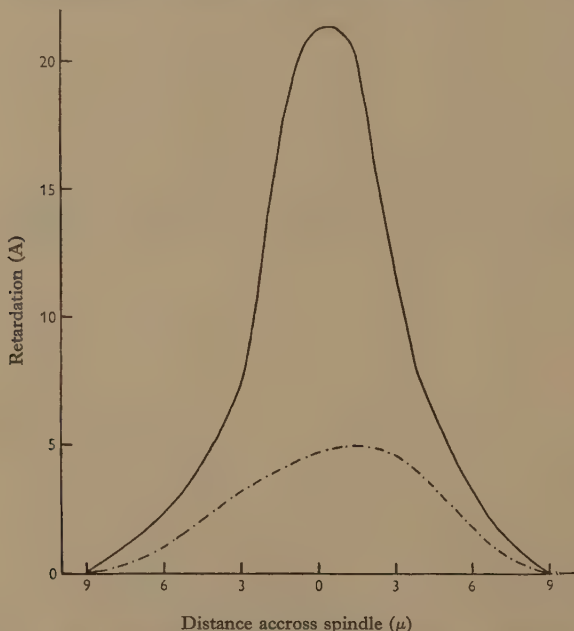
Text-fig. 5. Curve of retardation (continuous line) and coefficient of birefringence (dashed line) of the metaphase spindle, in Pl. 8, fig. 1. The inset shows the shape of spindle assumed for the coefficient of birefringence calculations.



Text-fig. 6. The growing retardation of the spindle in prophase and metaphase. These curves are taken from figs. 4-6 and fig. 1 of Pl. 8, 50, 52, 54 and 56 min. after fertilization.

coefficient of birefringence. In Text-fig. 5 the curve of retardation of metaphase spindle is compared with the curve for coefficient of birefringence calculated in this way. As with the aster, there is a definite similarity between the two curves.

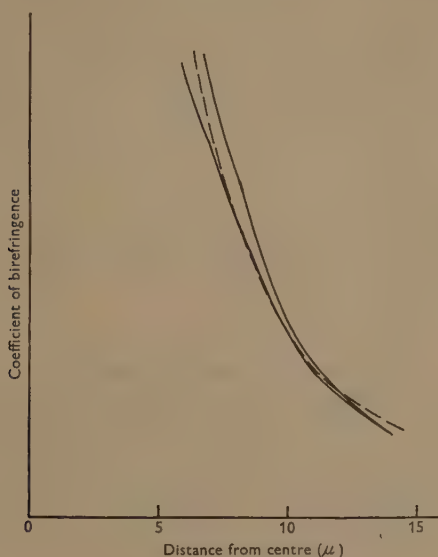
The calculation of coefficient of birefringence involves assuming a shape for the spindle (inset in Text-fig. 5), and allowing, as in the aster, for the fact that different elements of the spindle make different angles with the optical axis. The assumption of a definite shape is not likely to lead to serious error, but it is not strictly justified, as curves of retardation across the equator of the spindle show (Text-fig. 7). The spindle is evidently not clearly demarcated in this region, since the birefringence shades off gradually. Some allowance should also be made for birefringence outside the arbitrary limits of the spindle. This can be done, though it turns out that the correction is negligible, amounting at the most only to 5%.



Text-fig. 7. Curves of retardation across the equator of a spindle: at metaphase (continuous line) and 2 min. after the beginning of anaphase (dots and dashes).

The interpretation of the coefficient of birefringence curve for the spindle follows the same lines as for the asters. An examination of living cells and stained sections in ordinary light, and of unstained sections in phase contrast, shows that there are no significant variations of density within the spindle, so that once again, the fall in coefficient of birefringence must be due either to molecular and micellar changes or to a fall in the proportion of oriented material. A comparison of the fall in coefficient of birefringence with an inverse square is made in Text-fig. 8, and it is apparent that

there is close agreement. Once again, therefore, it seems possible that there is a structure of discrete submicroscopic fibrils radiating from the centrosomes. The oriented structure of the spindle, however, does not spread out freely into the cytoplasm as in the case of the asters, so that the coefficient of birefringence would hardly be expected to fall off quite as rapidly as an inverse square. Some of the orientation at the equator, moreover, is presumably due to the centromeres. The close agreement between the calculated curve and an inverse square may therefore be a little misleading, and it is likely that, but for these modifying factors, the coefficient of birefringence would fall off rather more rapidly than an inverse square, as it does in the case of the aster.



Text-fig. 8. Curves of coefficient of birefringence for each half spindle taken from Text-fig. 4 (continuous lines), and an inverse square curve (dashed line).

SUMMARY

1. The present paper is the first of a series dealing with the birefringence of mitotic figures in the eggs of the sea-urchin *Psammechinus miliaris*.
2. Living eggs have been examined using time-lapse photography, and retardation curves for the mitotic figures constructed from densitometric measurements made on the film negatives.
3. In the case of the aster, an integral equation relating retardation and coefficient of birefringence can be formulated and solved exactly to give coefficient of birefringence. In the case of the spindle, coefficient of birefringence can only be calculated approximately.
4. In both asters and spindles, the coefficient of birefringence is nil at the centres,

risers to a maximum at 5 or 6 μ out, and then falls to a minimum at the equator of the spindle or the periphery of the aster.

5. The rise in coefficient of birefringence round the centre is not as sharp as might be expected, and there is some evidence that orientation is built up gradually over a distance of a few microns.

6. The fall in coefficient of birefringence away from the maximum is approximately an inverse square in the case of the spindle. In the aster it falls off somewhat more rapidly. Since the density of material does not vary from point to point, this fall must be due to changes in molecular and micellar arrangement, or to a decreasing proportion of oriented material.

7. The classical conception of the spindle and asters as structures built up of discrete fibrils radiating from the centres, would be expected, for geometrical reasons, to give an inverse square fall in proportion of oriented material. While, therefore, a homogeneous structure with varying molecular and micellar arrangement cannot be ruled out, it is possible that the mitotic figure consists of definite fibrils radiating from the centres.

8. Evidence from other sources supports this view, and suggests that the fibrils must be submicroscopic in size.

I should like to thank the Director and Staff of the Marine Station, Millport, Scotland, for their help on many occasions.

APPENDIX I

An outline of polarized light microscopy

A polarizing microscope is essentially only an ordinary microscope fitted with Nicol prisms or sheets of Polaroid. One prism or Polaroid is fitted below the condenser, and is known as the polarizer; another, known as the analyser, is fitted above the objective. Polarizing materials only transmit light vibrating in a particular plane, so that if the plane of the analyser is set at right angles to the plane of the polarizer, the microscope will transmit (theoretically at least) no light at all.

Consider now the effect of a birefringent object between the polarizer and analyser. Light traversing the object can be considered as being split up into two components, vibrating in planes at right angles to each other; but since in birefringent materials, the refractive index is not the same for light vibrating in different planes, the two components will travel at different speeds. When they emerge from the object, therefore, they will be to some extent out of phase, and instead of recombining to give a plane-polarized beam, they will produce 'elliptically' polarized light. A component of this light will vibrate parallel to the plane of the analyser, and will therefore get through to the field of the microscope.

The measure of birefringence is the extent to which the two components are out of phase when they emerge from the object. This is normally referred to as the retardation, and is measured in wave-lengths. In the present work, however, the retardations are so small that it is more convenient to measure them in Angstroms (wave-length of green light = 5500 Å.).

Retardations are caused, as we have seen, by objects whose refractive index is not the same for light vibrating in different planes. This difference in refractive index is known as the coefficient of birefringence ($n_e - n_o$). Retardation and coefficient of birefringence are very simply related: the retardation of an object is the product of its coefficient of birefringence and its thickness. Thickness and retardation must, of course, be measured in the same units.

In most oriented biological structures, including mitotic figures, there are only two planes with different refractive indices, and these coincide with the ordinary axes of symmetry of the object, e.g. in the case of a fibre, parallel to and at right angles to its length. Birefringence may be either positive or negative with respect to a particular axis, depending on whether the refractive index parallel to that axis is the greater or lesser one. It is usual to refer the sign of birefringence of an object to its long axis.

The retardation produced by an object is a function of the angle between its axes and the planes of the polarizer and analyser. At 45° the retardation is at a maximum, while at 0 or 90° it falls to nil. For this reason birefringent objects are normally examined with their axes in the 45° position, and this has been done in all the photographs of the present work.

Much use is made, in polarized light microscopy, of a birefringent plate, or compensator, which is inserted between the polarizer and analyser, usually just above the polarizer. By this means, stray birefringence in lenses or slides can be counteracted, and strength and sign of birefringence can be determined. The compensator can also be used to increase the contrast of weakly birefringent objects (Swann & Mitchison, 1950). Because it adds either positive or negative birefringence to the whole field of the microscope, the compensator can make birefringent objects appear brighter or darker than the background. For the same reason it causes radially symmetrical objects like asters to appear bright in two quadrants and dark in the other two. This effect is evident in all the photographs of Pl. 8.

We have seen that both strength and sign of birefringence may vary. In addition, there are two distinct types of birefringence, known as form and intrinsic. Intrinsic birefringence is due solely to molecules, whereas form birefringence is produced by larger aggregates such as micelles. Form birefringence depends simply on the presence of asymmetrical particles of different refractive index from the surrounding medium, the particles themselves need not be birefringent. The majority of biological tissues show both types of birefringence; the relative contributions can usually be distinguished by immersing the tissue in a medium of high refractive index, when the form birefringence disappears.

A full account of the theory and practice of polarized light microscopy is given by Bennett (1950).

APPENDIX 2

The derivation of retardation from measurements of film density

The derivation of retardation from densitometer measurements, presents certain difficulties in the case of the sea-urchin egg. Normally there are two possible methods: either to calibrate the negative in terms of retardation, or to work from the characteristic curve of the film. In the first case it is only necessary to include a

number of blank frames at the end of a run, each illuminated with known amounts of retardation produced by means of a compensator. A curve relating retardation to the densitometer readings can then be constructed. In the second case, a number of blank frames are included in the same way, but with a constant setting of the compensator, and with exposure increasing in multiples. From these blank frames, the characteristic curve of the film can be constructed, and densitometer readings converted to light intensity. Since retardation is proportional to the square root of intensity, for small retardations, it is a simple matter, after converting the intensity values to a linear scale and establishing a zero line, to calculate retardation.

If the object scatters no light, the first of these methods is by far the simpler. The granules in the sea-urchin egg, however, scatter a certain amount of light even at low N.A.'s. This has the effect of raising slightly the whole level of the densitometer curves, and so making it impossible to read off retardation directly from a calibration curve.

The difficulty can be overcome using either method. In the second case it happens automatically, since a zero line has to be drawn; this line is simply drawn rather higher than would otherwise be the case. Using the first method it becomes necessary to draw a zero line, and subtract from the densitometer readings the amount by which the zero differs from the background. For this, of course, the densitometer readings must be converted to a linear scale. Since all the photographs of living asters and spindles are taken with a compensator in position, it is necessary, in both cases, to subtract the amount of compensation from the final figure. The effect of allowing for light scattering is to make the first method as long as the second, and in fact both of them have been used in working out the results. It should be added that they give the same answer.

These methods, though laborious, present no difficulty apart from the establishment of the zero line. In the case of the spindle this is simple, since it only involves drawing a straight line from the minimum of one centre, to the minimum of the other. This line should be horizontal, although occasionally the whole background may have a slight gradient, if the field illumination is not quite even. The zero line for asters is not so easily established. If there were no light scattering this also would be horizontal, but in fact the light scattered is a function of the optical section of the egg, so that the zero line is necessarily slightly tilted, and is strictly speaking not a straight line, but a curve. In practice, the curve is so gradual that a straight line is a reasonable approximation. Occasionally there is some doubt as to how this line should be drawn, though it is usually obvious. The precise position of the line has very little effect on the proximal parts of the aster, but affects the distal regions rather more seriously; hence the uncertainty referred to in the text about the precise values for retardation at the edge of the aster.

The only remaining operation is the smoothing of the retardation curves. The strongly birefringent regions of the spindle and asters are in fact so smooth that it is only necessary to draw a line through all the points. The peripheral regions of the asters and spindles in late anaphase, however, are liable to be bumpy. These bumps are not usually greater than $0.5\text{ \AA}$., so that smoothing is not difficult.

APPENDIX 3

The integral equation of aster structure and its solution

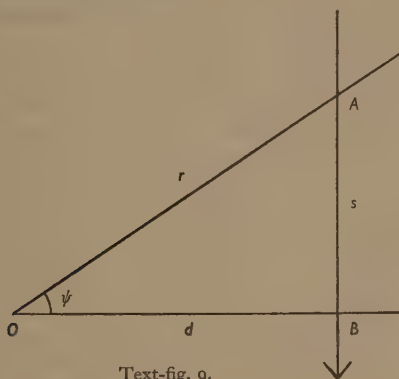
O is the centre of an aster;

AB is the axis of vision;

$C_{(r)}$ is the coefficient of birefringence at any point in the aster, at distance r from the centre;

$T_{(d)}$ is the retardation of the aster at distance d from the centre, seen in plan view, due to birefringent elements along the AB axis;

s and ψ are as shown in Text-fig. 9.



Text-fig. 9.

If an oriented element, with $(n_e - n_o)$ small, lies with its optical axis at an angle θ to a ray, it can be shown by taking the polar equation for its velocity ellipsoid, that its effective birefringence along the ray path is $(n_e - n_o) \sin^2 \theta$. Thus:

$$T(d) = \int_0^\infty C_{(r)} \cos^2 \psi \, ds \quad (r = d \sec \psi, s = d \tan \psi, ds = d \sec^2 \psi \, d\psi). \text{ Therefore}$$

$$T(d) = \int_0^{\frac{1}{2}\pi} C(d \sec \psi) \cos^2 \psi \, d \sec^2 \psi \, d\psi = d \int_0^{\frac{1}{2}\pi} C(d \sec \psi) \, d\psi.$$

The solution of this equation, for which I am indebted to Mr Freeman Dyson, is

$$C_{(\rho)} = \frac{2}{\pi} \int_0^{\frac{1}{2}\pi} \left[\frac{2T(\rho \sec \psi)}{\rho \sec \psi} - T'(\rho \sec \psi) \right] \cos \psi \, d\psi,$$

where $C_{(\rho)}$ is the coefficient of birefringence at distance ρ from the centre. The practical application of this solution involves first plotting a smooth retardation curve, and measuring the slope, $T'_{(r)}$, for different values of r . Values of the function $R_{(r)}$ are then calculated and plotted against r .

$$R_{(r)} = \left[2 \frac{T_{(r)}}{r} - T'_{(r)} \right].$$

Then

$$C_{(\rho)} = \frac{2}{\pi} \int_0^{\frac{1}{2}\pi} R(\rho \sec \psi) \cos \psi d\psi.$$

Values for $C_{(\rho)}$ for a sufficient number of values of ρ are worked out by numerical integration.

APPENDIX 4

The limitations of the optical system

Sharp changes of light intensity in the object are spread out in the image, to an extent determined primarily by the N.A. of the objective. All the photographs in the present work were taken with a $10\times$ objective of N.A. 0.28. Under these conditions a sharp edge is spread out to a more or less steady slope, the top and bottom of which are separated by about 2μ . Gradients of intensity in the object that are as steep as this will not therefore be accurately reproduced. The steepest gradients to be found in the retardation curves of the present work are those between the centres and the maxima of the asters and spindles. The distance between top and bottom, however, is about 5 or 6μ , so that the transitions are well within the capacity of the system.

A possible error is concealed in the assumption that the retardation measured at a particular point is the true retardation along the optical axis at that point. In fact, the light that traverses the object is not parallel, but in the form of a cone, whose angle is determined by the aperture of the condenser. As a result the marginal rays cut the aster or spindle slightly nearer the centre. The retardation as measured at any point is effectively therefore the retardation of a point slightly nearer the centre, so that the whole retardation curve is displaced outwards. The degree of displacement, however, is very slight, being only about 0.06μ at 15μ from the centre.

The last difficulty concerns depth of focus. It has been tacitly assumed that light from every point along the optical axis comes to a focus at the same point in the image plane. This, however, is only the case over a certain range known as the depth of focus. Unfortunately there is no entirely satisfactory formula for depth of focus, the standard quarter wave-length criterion of Rayleigh being generally recognized as too stringent. On this criterion, the depth of focus at the N.A. used in this work (0.28) would be about 10μ . While it is desirable that the whole object should fall within this distance, it is not essential, and provided that the transitions are not sharp, the object can be considerably out of focus, and yet be reproduced with reasonable accuracy.

The sharp transitions of light intensity in the spindle and asters are to be found in two different regions. The first is between 2 and 4μ from the centres, where retardation is building up rapidly. This transition clearly falls well within the depth of focus limit, even on the Rayleigh criterion. The second region, where the transitions are rather less sharp than in the first, falls between about 5 and 8μ in the aster, and 6 and 12μ in the spindle. To reproduce the aster perfectly would therefore need a depth of focus of about $2 \times 8 = 16\mu$. But, as already pointed out, the main retardation is caused by elements lying more or less normally to the optical axis. Calculation shows in fact that the contributions of elements making an angle of less than 45° to the optical axis is negligible. Under these circumstances the required depth of focus is about $2 \times 8 \sin 45^\circ = 11\mu$. The aster is therefore likely to be reproduced

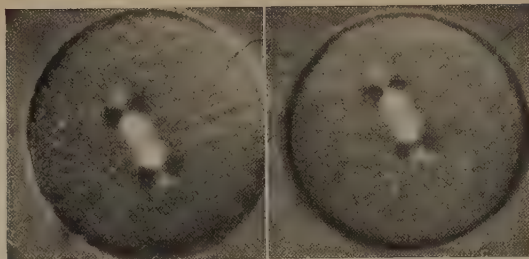


Fig. 1.

Fig. 2.

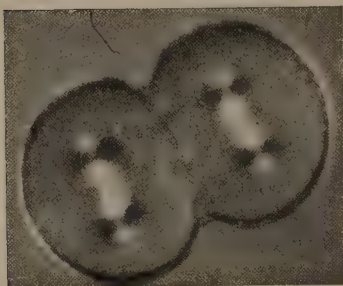


Fig. 3.

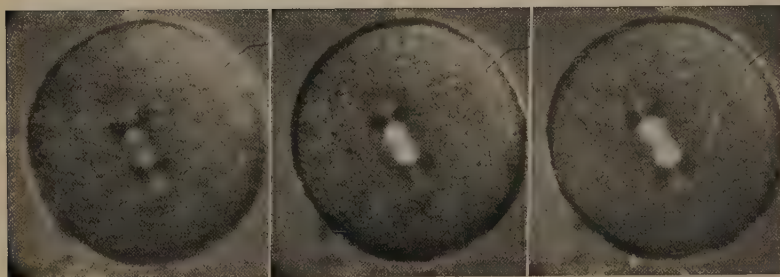


Fig. 4. 50 min.

Fig. 5. 52 min.

Fig. 6. 54 min.

fairly accurately by the present system. The spindle is a slightly different case, since it is not radially symmetrical about the centres. The traverses across the spindle in Text-fig. 7 show that its maximum breadth is about 18μ , but the outer regions are not strongly birefringent. Most of the retardation lies within 5μ of the axis, so that once again a depth of focus of 10μ should be adequate. The correctness of these conclusions is borne out by the simple experiment of focusing up and down on a mitotic figure. Over a wide range there is no perceptible difference in appearance.

Although the various effects inherent in the optical system are not likely to be serious, they necessarily all tend towards smoothing out steep gradients in the true curves of retardation. For this reason it is unwise to place great reliance on the absolute values and precise shapes of the measured retardation curves. It is difficult, for instance, to be certain that the rather gradual rise in retardation round the centrosomes is not an artefact, though it seems unlikely. There can be no doubt, however, about the general shape of the curves.

REFERENCES

- BENNETT, H. S. (1950). The microscopical investigation of biological materials with polarized light. In McClung's *Handbook of Microscopical Technique*, 3rd ed. New York.
- CHAMBERS, R. (1924). The physical structure of protoplasm as determined by micro-dissection and injection. In Cowdry's *General Cytology*. Chicago.
- CORNMAN, I. (1944). A summary of evidence in favour of the traction fiber in mitosis. *Amer. Nat.* **78**, 410.
- HEILBRUNN, L. V. (1943). *Outline of General Physiology*, 2nd ed. Philadelphia.
- HUGHES, A. F. W. & SWANN, M. M. (1948). Anaphase movements in the living cell. A study with phase contrast and polarized light on chick tissue cultures. *J. Exp. Biol.* **25**, 45.
- INOUE, S. (1950). Personal communication.
- KUWADA, Y. & NAKAMURA, T. (1934). Behaviour of chromonemata in mitosis. IV. Double refraction of chromosomes in *Tradescantia reflexa*. *Int. J. Cytol.* **6**, 78.
- MITCHISON, J. M. (1952). Cell membranes and cell-division. *Symp. Soc. Exp. Biol.* **6** (in the Press).
- MONNÉ, L. (1944). Cytoplasmic structure and cleavage pattern of the sea-urchin egg. *Ark. Zool.* **35A**, no. 13.
- RIS, H. (1943). A quantitative study of anaphase movement in the aphid *Tamalia*. *Biol. Bull. Woods Hole*, **85**, 164.
- ROZSA, G. & WYCKOFF, R. W. G. (1950). The electron microscopy of dividing cells. *Biochem. Biophys. Acta*, **6**, 334.
- RUNNSTRÖM, J. (1928). Die veränderung der Plasmakolloide bei der Entwicklungserregung des Seeigeleies. *Protoplasma*, **4**, 388.
- SCHMIDT, W. J. (1937). *Die Doppelbrechung von Karyoplasma, Metaplasma und Zytoplasma*. Berlin.
- SCHMIDT, W. J. (1939). Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. *Chromosoma*, **1**, 253.
- SCHRADER, F. (1944). *Mitosis*. New York.
- SWANN, M. M. (1951*a*). Protoplasmic structure and mitosis. II. The nature and cause of the birefringence changes in the sea-urchin egg at anaphase. *J. Exp. Biol.* **28**, 434.
- SWANN, M. M. (1951*b*). Structural agents in mitosis. *Int. Rev. Cytol.* **1** (in the Press).
- SWANN, M. M. (1952). The nucleus in fertilisation, mitosis and cell-division. *Symp. Soc. Exp. Biol.* **6** (in the Press).
- SWANN, M. M. & MITCHISON, J. M. (1950). Refinements in polarized light microscopy. *J. Exp. Biol.* **27**, 226.
- SWANN, M. M. & MITCHISON, J. M. (1951). Birefringence of cytoplasm and cell membranes. *Progress in Biophysics*, **2**.

EXPLANATION OF PLATE 8

Figs. 1-3. Metaphase mitotic figures of *Psannechinus miliaris*, in polarized light. $\times 400$. Compensated.

Figs. 4-6. Growth of the mitotic figure in prophase. $\times 400$. Compensated. Photographs taken at 50, 52 and 54 min. after fertilization. Fig. 1 is the 56 min. stage of this series.

PROTOPLASMIC STRUCTURE AND MITOSIS

II. THE NATURE AND CAUSE OF BIREFRINGENCE CHANGES IN THE SEA-URCHIN EGG AT ANAPHASE

By M. M. SWANN

*From the Department of Zoology, University of Cambridge,
and the Marine Station, Millport*

(Received 15 March 1951)

(With Plates 9 and 10 and Twelve Text-figures)

INTRODUCTION

It is well known from the work of Schmidt (1937, 1939) that the birefringence of the mitotic figure of the sea-urchin egg decreases during anaphase. The same effect was also noticed in chick tissue culture cells by Hughes & Swann (1948). By analogy with the contraction of muscle fibres and protozoan myonemes, Schmidt interpreted this decrease as evidence for the contraction of spindle fibres. Whether or not his interpretation is correct, however, it is clear that extensive changes in protoplasmic structure do take place during anaphase. The present paper describes one aspect of these changes.

In the first paper of this series (Swann, 1951*a*), the technique of quantitative birefringence analysis for spindles and asters was described. The retardation as measured at any point in the mitotic figure is the sum of the retardations of all the elements of the spindle or aster traversed by the illuminating beam at that point. To interpret structure, however, it is preferable to know the coefficient of birefringence within the spindle or aster, rather than the total retardation. In the case of the aster, because of its radial symmetry, a curve for coefficient of birefringence can be calculated exactly from the retardation data. In the case of the spindle, however, a curve for coefficient of birefringence can only be calculated by assuming a definite shape; but as the spindle is neither clearly defined, nor constant in length, it is usually more satisfactory to compare curves of retardation. The two types of curve, in any case, are rather similar. With the asters, of course, there is no difficulty about comparing curves for coefficient of birefringence.

In comparing the curves for different stages in anaphase, both increases and decreases of birefringence are apparent. The molecular interpretation of these changes is an interesting problem, but not, as will become evident, immediately relevant to the question of what is causing the changes. It can, in fact, only be considered in relation to the form and intrinsic birefringence of the mitotic figures, and this raises further difficulties, since fixatives and imbibition agents all affect the labile protoplasmic structures of the egg. A detailed examination of these questions is left to a later paper.

SPINDLE CHANGES DURING ANAPHASE

The photographs in Pl. 9, figs. 1-4, are taken from a time lapse film (series A) of an egg of *Psammechinus miliaris*. They show the egg in metaphase, and at three successive stages in anaphase. It is evident that the birefringence of the whole mitotic figure decreases steadily, while the asters grow and the spindle lengthens. Because of this lengthening, complete spindle retardation curves cannot be superimposed, and the polar and equatorial regions have to be compared separately. This is done in Text-figs. 1 and 2, the curves of which are derived from the photographs in Pl. 9, figs. 1-4.

The first retardation curve, 56 min. after fertilization, shows the spindle in metaphase. It should be compared with the 58 min. curve. At the poles, both curves fit precisely, and there has evidently been no change in structure there during the interval of 2 min. At the equator, however, there has been a considerable decrease in retardation, and therefore in coefficient of birefringence. The next curve, at 60 min., shows a further decrease at the equator, and a slight decrease near the poles but a greater decrease midway between the two. The fourth curve, at 62 min. (shortly before cleavage), shows little change at the equator, but a further decrease in the polar and subpolar regions.

These changes are characteristic. They can be followed in more detail in series B, where the time intervals are shorter. Photographs from this series are shown in Pl. 9, figs. 5-10, and the corresponding retardation curves are given in Text-figs. 3 and 4. Four photographs of an egg in the two-cell stage during anaphase, showing similar changes, are given in Pl. 9, figs. 11-14.

From these curves and photographs, it is evident that the decrease in coefficient of birefringence first appears at the equator of the spindle. Two regions of decreasing birefringence then move away from the equator towards either pole. In the final stages, the equatorial birefringence remains unchanged, and the decrease is confined to the region near the poles. This sequence of events is shown diagrammatically in Text-fig. 5, where the region of the spindle that shows a decrease in birefringence is plotted against time. The region in question is shown as a vertical line stretching the appropriate distance, the scale is being measured in microns from the equator. The gradual movement of the region of decreasing birefringence from the equator to the poles is obvious. These changes have been photographed in many dozens of eggs, and analysed quantitatively in seven cases. The same sequence of events is always apparent.

The pattern of birefringence decrease during anaphase closely resembles the movements of the chromosomes. They also start at the equator, and move apart in two groups towards either pole. The next step, therefore, is clearly to find out whether the region of decreasing birefringence actually corresponds to the position of the chromosome groups. In the sea-urchin egg unfortunately, this is not easily decided, for the chromosomes are invisible by ordinary light, by phase contrast, or by polarized light. They can, of course, be seen in fixed and stained specimens, but the birefringence of the spindle is then drastically altered.

The problem was finally solved by putting single living eggs in a drop of sea water on a slide, and following their development under the polarizing microscope with the time-lapse camera. When the eggs reached particular stages they were fixed, dehydrated, and embedded in a drop of celloidin. The drops of celloidin were hardened, embedded in wax, and then sectioned and stained in the ordinary way. The position of the chromosomes in the spindle was then determined, and correlated with the appearance of the egg in polarized light just before fixation, and with the region of birefringence decrease as measured from the time-lapse film.

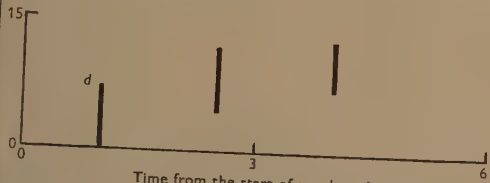
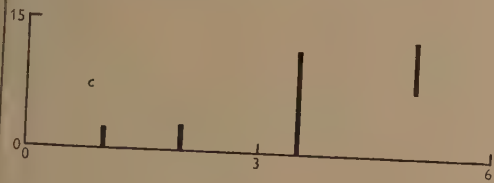
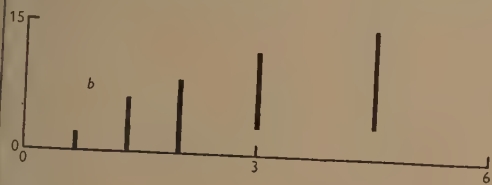
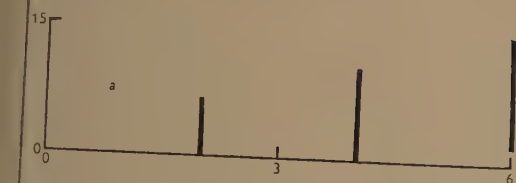
About thirty single eggs were treated in this way, and the results leave no doubt that the region of birefringence decrease does correspond to the position of the chromosome groups. A few of the results of this investigation are shown in Pl. 10, where a diagrammatic representation of the position of the chromosomes in the spindle, determined from sections, is shown beneath the photographs of the egg just before fixation. The approximate positions of the chromosomes, estimated from these data, have been inserted in the spindle retardation curves of Text-figs. 1 and 3.

The fact that the region of birefringence decrease is extensive, while the chromosome groups are compact, suggests that the decrease is not due directly to the chromosomes, as it might be if they were large and possessed a sign of birefringence opposite to the spindle material. Sea-urchin chromosomes in fact, appear to be completely isotropic. The conclusion is inevitable that the decrease in birefringence is due to structural changes in the spindle material. The fact that the decrease occurs only in the regions round the chromosomes, suggests that the structural changes are actually initiated by them.

ASTER CHANGES DURING ANAPHASE

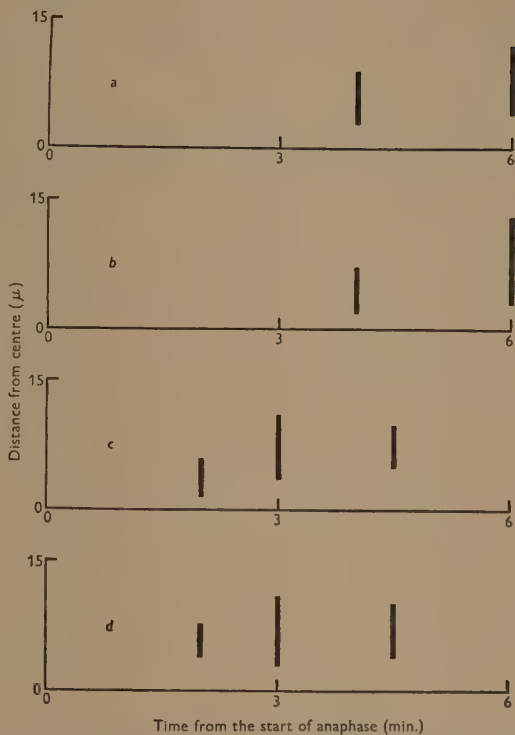
Curves for coefficient of birefringence in the asters of series A are given in Text-figs. 6 and 7. The form of the curves at metaphase (56 min.) has already been discussed (Swann, 1951*a*). At this stage the asters only extend out to about 15μ . By 58 min., however, there has been a marked increase in coefficient of birefringence in all but the most central regions of the asters, as well as an increase in radius of about 3μ . By contrast, the spindle at the same stage shows only a slight drop in the region of the equator (Text-fig. 1). By 60 min., however, the familiar decrease in birefringence has begun, the main region of decrease being between 3 and 6μ . Further out however, beyond about 9μ , there has been a slight increase in birefringence, and the asters have grown to about 21μ . By 62 min. there has been a further decrease in birefringence, this time between 6 and 12μ , and a further slight increase beyond 15μ . The asters have grown to nearly 30μ in radius.

The anaphase changes in the spindle appear to consist mainly of a shifting pattern of birefringence decrease. In the asters, on the other hand, there are clearly two separate processes at work. The first of these, which starts at the very beginning of anaphase, is a gradual increase in the coefficient of birefringence of all but the innermost regions of the asters. This leads to a steady growth in actual size. The second process, which does not become apparent until at least 2 min. after the start of anaphase, is a decrease in the coefficient of birefringence. Unlike the increase just



Time from the start of anaphase (minutes)

agrams to illustrate the way in which the region of birefringence drop moves equator to the poles of the spindle during anaphase. The vertical lines the regions in which there has been appreciable drop, averaged from both the spindle. *a*, series A; *b*, series B; *c* and *d*, taken from two cell stages, of which are not reproduced.



Text-fig. 10. Diagrams to illustrate the way in which the region of birefringence drop in the aster moves outwards from the centrosome during anaphase. The vertical lines represent the regions in which there has been appreciable drop. *a*, series A, upper aster; *b*, series A, lower aster; *c*, series B, upper aster; *d*, series B, lower aster.

discussed, this decrease does not affect the whole aster at the same time. It starts from the centre and moves outwards. It affects not merely the more birefringent regions of the original metaphase asters, but also the birefringence built up in the first few minutes of anaphase. It does not appear to affect the rather weak birefringence built up in the outer regions of the aster during anaphase, though there may of course be a decrease in these regions that is masked by a more rapid increase.

The final state of affairs is an aster of about double the radius and one-third the coefficient of birefringence of metaphase. The strength of birefringence is actually rather variable, and tends to be higher in two-cell than in one-cell stages. The final curve differs in various respects from the metaphase curve; its maximum is more flat-topped, and occurs rather further out from the centre.

These changes can be followed in more detail in Text-figs. 8 and 9, taken from series B. The shift of the regions of birefringence decrease with time, is shown diagrammatically in Text-fig. 10, which should be compared with the corresponding diagrams for the spindle (Text-fig. 5).

The most striking fact about the decrease of birefringence in the asters is that it does not begin until some 2 min. after decrease has begun in the spindle. By examining the various text-figures, however, it can be seen that this interval corresponds in fact to the time between the beginnings of birefringence decrease at the equator and at the poles of the spindle. The decrease of birefringence in the asters would seem therefore to be simply a part of the same process that is going on in the spindle.

Before considering the other aspect of the changes in the asters during anaphase, that is their growth in size, it is necessary to examine their behaviour in the earlier stages of the mitotic cycle. A cursory examination in ordinary light suggests that they reach a diameter of about $15\ \mu$ quite early in prophase, and then remain constant in size until anaphase. A more detailed examination in polarized light bears out this conclusion. In Text-fig. 11, for example, the radius of the asters in series B is plotted against time. On the same graph the retardation of the spindle equator is plotted to show the start of anaphase. The aster radius remains more or less constant at $15\ \mu$ up to the start of anaphase, when it begins to increase rapidly. The same effect is shown even more clearly in Text-fig. 12, where aster radius, measured on fixed and stained cells, is correlated with the state and position of the chromosomes. Throughout prophase and metaphase the aster radius remains constant, but as soon as the chromosomes start to separate it rises sharply, reaching, by the end of anaphase, a value about double that at metaphase. It is evident that the growing aster very soon reaches some sort of equilibrium, which is maintained throughout prophase and metaphase. With the onset of anaphase, however, this equilibrium is upset, and the aster starts once more to grow.

At first sight there appears to be no counterpart in the spindle, to the growth of the asters during anaphase. Curves of retardation across the spindle equator show no increase in diameter during anaphase (Swann, 1951*a*), nor is there at any time, a marked increase in coefficient of birefringence. On the other hand, there must be a certain intake of new material since the spindle as a whole, elongates. Why the intake

of extra material should lead to elongation has been discussed elsewhere (Swann, 1951*b*), and need not concern us here. It is interesting, however, to find that elongation, like aster growth, starts at the beginning of anaphase. In Text-fig. 11, the length of the spindle in series B is plotted against time. Although there is a slow elongation during prophase and metaphase, it is evident that the rate of elongation rises markedly at the beginning of anaphase. The same effect is seen more strikingly in the cells of the chick, where spindles may remain static for many minutes, but elongate suddenly as the chromosomes start to move apart (Hughes & Swann, 1948). Similar effects have been noticed by Ris (1943, 1949) and Bonnevie (1947). Evidently the spindle, like the asters, is in a condition of equilibrium during metaphase, but

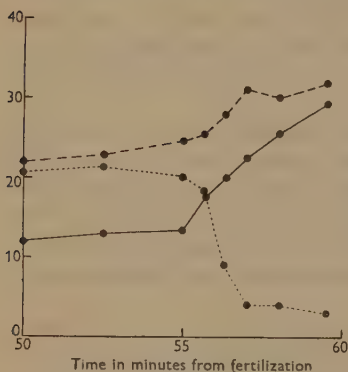


Fig. 11.

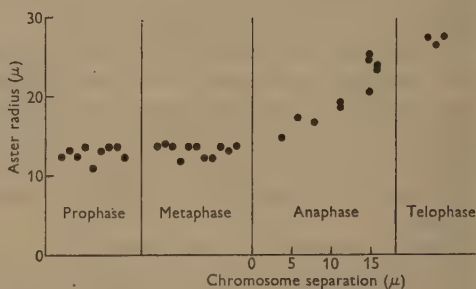


Fig. 12.

Text-fig. 11. The variation of aster radius (average for both asters), spindle length, and retardation of the spindle equator with time, from the data of series B. The sharp drop in retardation of the spindle equator at about 55 min. marks the beginning of anaphase. Retardation of spindle equator (A), short dashes; spindle length (μ), long dashes; aster radius (μ), continuous line.

Text-fig. 12. The relation between aster radius and the state and position of the chromosomes, taken from fixed and stained specimens. The spacing of the points in prophase, metaphase and telophase is arbitrary; in anaphase, aster radius is plotted against chromosome separation.

with the start of anaphase becomes capable of absorbing fresh material. Whereas, however, the aster simply increases in coefficient of birefringence, and grows in diameter, the spindle, because of its particular shape and structure, grows in length.

In short, while metaphase is a static period, there are two quite distinct types of structural change that affect the mitotic figure in anaphase. The first of these is a decrease of birefringence, which starts round the chromosomes as they leave the metaphase plate, moves with them to either spindle pole, and then spreads slowly outwards in the asters. The second change is a gradual growth that affects the whole mitotic figure at the same time, appearing in the spindle as an increase in length, and in the asters as an increase in size.

THE SIGNIFICANCE OF THE BIREFRINGENCE CHANGES

The metaphase spindle and asters both show a fall in coefficient of birefringence with distance from the centrosome. The significance of this was discussed in a preliminary way in the first paper of the present series (Swann, 1951*a*). It was argued that since there are no marked variations of density within the asters and spindle, the fall in coefficient of birefringence must be due either to changes in the proportion of oriented material, or to changes in molecular and micellar arrangement. The fall in coefficient of birefringence with distance, however, is very approximately an inverse square. It may possibly be explained therefore by a structure of discrete fibrils radiating from the centrosome, which, for geometrical reasons, would give an inverse square fall in proportion of oriented material. It is also possible, however, that the aster and spindle are homogeneous structures, in which case the fall in coefficient of birefringence must be accounted for solely in terms of changes in molecular arrangement. Further discussion of these two structures is not possible without a consideration of form and intrinsic birefringence, which raises a number of difficult problems. The whole question was therefore left to a later paper.

In view of this uncertainty about the significance of the variation of coefficient of birefringence with distance, it is clearly useless to attempt a detailed interpretation of the changes in coefficient of birefringence with time. A preliminary discussion, however, is not perhaps without value.

The fact that, throughout anaphase, the asters are growing in diameter, must mean that fresh material is being oriented. The proportion of oriented material, in other words, is increasing at $15\ \mu$ from the centre and beyond, from nil to some finite value. The coefficient of birefringence, however, is also increasing within the limits of the original metaphase aster. This may be due to changing molecular and micellar arrangements, but it is tempting to suppose that it also is due to an increase in the proportion of oriented material. This is borne out by the fact that the general shape of the curve for coefficient of birefringence does not appear to be altered at 1 or 2 min. after the start of anaphase; it is simply shifted outwards. It is perhaps significant also that the maximum value of the metaphase curve is not markedly exceeded during anaphase; a rather larger region of the aster merely attains the maximum value, suggesting perhaps that over this region all the available material is oriented.

The decrease in coefficient of birefringence, like the increase, might be due either to changes in the proportion of oriented material or to changes in molecular and micellar arrangement. The inner regions of the aster, however, suffer as much as a tenfold decrease in coefficient of birefringence, and the spindle even more. Yet the asters and spindle still retain their fibrillar appearance. It seems likely, therefore, that the decrease in coefficient of birefringence is due to molecular and micellar changes rather than an extensive decrease in the proportion of oriented material. This is essentially the conclusion of Schmidt (1937, 1939), who supposed the decrease to be due to a contraction of the fibres. Contraction necessarily involves molecular and micellar rearrangements, in which the various participating elements move from a predominantly longitudinal to a more random or even predominantly transverse arrangement. Such a change is bound to lead to a reduction in positive birefringence.

THE CAUSE OF THE BIREFRINGENCE CHANGES

In an earlier section, the close correlation between the position of the chromosomes and the regions of decreasing birefringence was pointed out. It was shown that the decrease could not be due directly to the chromosomes, which are small, and apparently isotropic. The region of decrease, on the other hand, is large, and spreads out on either side of the chromosome groups; moreover, when the chromosomes reach the neighbourhood of the centrosome, the birefringence decrease spreads out into the asters. It seems possible, therefore that the change is brought about indirectly by the chromosomes themselves.

Very little is known of how such structural changes as have been described in the present paper might be brought about. There would seem to be essentially two possible mechanisms. The changes might be the result of a self-propagating process, either physical or chemical, initiated by the chromosomes; or they might be caused by the release and diffusion from the chromosomes of substances, acting directly or indirectly on the protoplasm. In spite of the small amount of evidence available, it is possible to decide between these two alternatives with some certainty.

A self-propagating mechanism, no matter of what type, continues after it is initiated, without regard to the original stimulus. Once started, for instance, a nerve-action potential is unaffected by whether the original stimulus is repeated or removed. The pattern of movement of a propagated change is therefore unaffected by any subsequent movement of the stimulating mechanism. This, however, is conspicuously not the case with the pattern of birefringence decrease in the spindle. The region of decrease moves with the chromosome groups, whereas, if the mechanism were a self-propagating one, it should spread outwards, if not concentrically, at least without regard to the movements of the chromosomes.

We are led therefore to the idea that the decrease in birefringence is brought about by the release of a substance which, it may be supposed, diffuses away from the chromosomes, and produces its effect as it goes. A difficulty about such an explanation, however, is that the observed rates of movement are so slow. The spread of the decrease in birefringence is not easily measured in the spindle, because of the complicated pattern of change, though it is clearly of the order of only a few microns per minute. In the asters the rate can be measured more exactly, and seems to be not more than 5 or 6 μ per minute. As an example of the expected rate of diffusion in a sea-urchin egg, we can take the calculations of Rothschild (1949), although he was concerned with a slightly different problem, namely the time course of diffusion from a point on the periphery of the egg. From his curves it can be seen that a point in the centre of the egg 50 μ from the periphery, would reach 80% of the final concentration in 5 sec., for a diffusion constant of about 0.07 cm.²/day corresponding to a molecular weight of about 30,000.

This discrepancy might be overcome if the diffusing substance were of virus-like dimensions, though this seems improbable. A more likely explanation is that the oriented structure of the spindle and aster is retarding diffusion. Rothschild's calculations were based on the assumption that the viscosity of cytoplasm is only a little above that of water, and for the unfertilized sea-urchin egg this is known to be

the case. In the spindle and asters, on the other hand, there is an oriented structure, and it has long been realized that the resistance of such structures to the passage of molecules rises very sharply as the molecule approaches the pore size of the gel. If the diffusing substance in question consisted of relatively large molecules, diffusion might well be enormously slowed down. Yet another possibility is that ordinary diffusion is not operative, but that there is diffusion with a trap action. In such a system, the molecules of diffusing substance are caught and bound by the protoplasmic structure, so that the diffusion front does not advance until all the traps in its way are filled. Under these conditions, the apparent rate of diffusion is governed by the rate of release of the diffusing substance.

The increase in coefficient of birefringence during anaphase is a rather different case from the decrease discussed in the previous paragraphs, since it takes place simultaneously throughout the mitotic figure, leading to spindle elongation and aster growth. It would seem, therefore, that although the change in question is not completed at once, the whole cytoplasm is affected in some way in a short space of time after the start of anaphase. This is borne out to some extent by birefringence and light scattering changes in the cortex which also appear immediately after the start of anaphase. These changes are described by Mitchison & Swann (1952).

As there is no sign of a gradually moving pattern of change in this case, it is not possible to decide between a self-propagating mechanism and diffusion. Since, however, we have already postulated a diffusion mechanism of some sort for the decrease in birefringence, it is perhaps simplest to think in terms of the release of a second substance, controlling the growth of the spindle and asters, and possibly also the structure of the cortex. Since this change spreads throughout the cell much more rapidly than the decrease in birefringence, the substance in question cannot possess a very large molecule, nor can it be subject to a trapped diffusion mechanism.

CONCLUSIONS

From the evidence of the present paper, a hypothesis can be constructed to account for a small part of mitosis. The essential facts are that a pattern of changes in birefringence can be observed in the mitotic figure during anaphase. One type of change, which can be related to the position of the chromosomes, takes the form of a progressive decrease in coefficient of birefringence, probably due to molecular and micellar re-arrangements. The second type of change, which cannot be related to the position of the chromosomes, takes the form of an increase in coefficient of birefringence, probably due to the orientation of fresh material. The hypothesis suggests that these changes are brought about by the release of two different substances from the chromosomes during anaphase. The interpretation of the birefringence changes, though an interesting problem, is not relevant to the hypothesis.

It is not proposed to examine this hypothesis in detail, since observations to be described in later papers have also to be taken into account. There are, however, a few points of some interest that are relevant to the idea. The first of these is the bearing of the hypothesis on theories of anaphase movement. Since chromosomes

normally all start to move at the same time, it has usually been supposed that they are controlled by some unspecified external agency. A few cases are known, however, where some chromosomes move apparently autonomously (Schrader, 1944). The present hypothesis can perhaps explain such cases, since it supposes the chromosomes to be responsible for their own movement, by controlling the structural changes in the spindle. It should be added that the birefringence evidence suggests, as Schmidt (1937, 1939) has already concluded, that the mechanism of movement is a contractile one.

A further interesting point is the parallel between this hypothesis, and the suggestion put forward by Rothschild & Swann (1949) and Rothschild (1949) to account for the structural changes in the sea-urchin egg at fertilization. They observed and measured a cortical change spreading round the egg from the point of sperm entry, taking some 20 sec., and concluded that it could best be explained by supposing the sperm head to release some active substance which diffused through the egg. The parallel between the release of substances from the sperm nucleus and the chromosomes is obvious.

The hypothesis may also be relevant to the problem of cell division. Since the decrease of birefringence spreads outwards at about 5 or 6 μ per minute, it should take about 10 min. to reach the cell surface. This is in fact about the time after the beginning of anaphase at which cleavage begins. Moreover, since the chromosomes end up at either pole of the spindle, the change would be expected to reach the poles of the cell first; and there is clear evidence, both from birefringence and dark ground observations, that this is the case (Mitchison & Swann, 1952). These ideas are expanded into a hypothesis of cell division by Swann (1952) and Mitchison (1952).

If the chromosomes do in fact release active substances, it is clearly of the greatest importance to discover what they are. Since the ratio of total chromosome volume to total cell volume in the sea-urchin egg is only about 1 : 10⁴, and as only a part of the chromosomes could presumably consist of the substances in question, it is hardly to be expected that ordinary cytochemical methods would be sensitive enough to detect substances being released. A number of such tests have in fact been tried, but without success. The inference is that these hypothetical substances must be catalytic in nature, and the term 'Structural agents' has been suggested for them (Swann, 1951c).

SUMMARY

1. The mitotic figure of the sea-urchin egg is most strongly birefringent at metaphase. During anaphase this birefringence decreases considerably, but the spindle and asters both grow in size. These changes have been investigated quantitatively by constructing curves of retardation and coefficient of birefringence across the mitotic figure, using techniques described in an earlier paper.

2. The decrease of birefringence in the spindle starts at the equator, and then moves, in the course of a few minutes, to either pole. Only when the decrease has reached the spindle poles does it begin in the asters, where it moves outwards from the centres.

3. These changes resemble the movements of the chromosomes, which also start at the equator in metaphase, and move in separate groups to the poles during anaphase. By examining single eggs in anaphase up to the moment of fixation, and then staining them to show the chromosomes, it is established that the regions of decreasing birefringence actually correspond to the position of the chromosomes.

4. Since the chromosomes are too small to be the direct cause of the decrease in birefringence, it is concluded that they are producing the decrease indirectly by initiating a structural change in the spindle and asters.

5. The possible mechanisms for this change are discussed. It is concluded that the chromosomes must be releasing an active substance, for which the term 'structural agent' is suggested.

6. The growth of the mitotic figure takes the form, in the spindle, of an increase in length, and in the asters, of an increase in size. It is accompanied by an increase in coefficient of birefringence, though this is to some extent masked by the decrease in birefringence referred to earlier.

7. The increase in coefficient of birefringence affects the whole mitotic figure from the very beginning of anaphase, and is not therefore relatable to the position of the chromosomes. For this reason it might be due to a number of different mechanisms, but as it starts at the same moment as the decrease in birefringence, it is tentatively assumed to be due to the release of a second 'structural agent'.

8. The increase in coefficient of birefringence is probably due to the orientation of new material. The decrease is more likely to be due to changes in molecular and micellar arrangement; it would be consistent with a contractile mechanism in the spindle.

9. The implications of these findings are discussed in a concluding section.

REFERENCES

- BONNEVIE, K. (1947). On the mechanics of mitosis. *J. Morph.* **81**, 399.
- HUGHES, A. F. & SWANN, M. M. (1948). Anaphase movements in the living cell. A study with phase contrast and polarized light on chick tissue cultures. *J. Exp. Biol.* **25**, 45.
- MITCHISON, J. M. (1952). Cell membranes and cell-division. *Symp. Soc. Exp. Biol.* **6** (in the Press).
- MITCHISON, J. M. & SWANN, M. M. (1952). In preparation.
- RIS, H. (1943). A quantitative study of anaphase movement in the aphid *Tamalia*. *Biol. Bull. Woods Hole*, **85**, 164.
- RIS, H. (1949). The anaphase movement of chromosomes in the spermatocytes of the grasshopper. *Biol. Bull. Woods Hole*, **96**, 90.
- ROTHSCHILD, LORD (1949). The fertilization reaction in the sea-urchin egg. A note on diffusion considerations. *J. Exp. Biol.* **26**, 177.
- ROTHSCHILD, LORD & SWANN, M. M. (1949). The fertilization reaction in the sea-urchin egg. A propagated response to sperm attachment. *J. Exp. Biol.* **26**, 164.
- SCHMIDT, W. J. (1937). *Die Doppelbrechung von Karyoplasma, Metaplasma und Zytoplasma*. Berlin.
- SCHMIDT, W. J. (1939). Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. *Chromosoma*, **1**, 253.
- SCHRADER, F. (1944). *Mitosis*. New York.
- SWANN, M. M. (1951*a*). Protoplasmic structure and mitosis. I. The birefringence of the metaphase spindle and asters of the living sea-urchin egg. *J. Exp. Biol.* **28**, 417.
- SWANN, M. M. (1951*b*). The Spindle, in Hughes' *The Mitotic Cycle*. London.
- SWANN, M. M. (1951*c*). Structural agents in mitosis. *Int. Rev. Cytol.* **1** (in the Press).
- SWANN, M. M. (1952). The nucleus in fertilisation, mitosis and cell division. *Symp. Soc. Exp. Biol.* **6** (in the Press).

EXPLANATION OF PLATES 9 AND 10

PLATE 9

Figs. 1-4. Series A. Photographs taken from a time-lapse film of an egg of *Psammechinus miliaris* in anaphase. $\times 300$. Compensated. The times after fertilization are shown beneath each photograph. Fig. 1 is at metaphase.

Figs. 5-10. Series B. Photographs taken from a time-lapse film of an egg of *P. miliaris* in anaphase. $\times 300$. Compensated. The times after fertilization are shown beneath each photograph. Fig. 5 is at metaphase.

Figs. 11-14. Photographs taken from a time-lapse film of a two-cell stage egg of *P. miliaris* in anaphase. $\times 300$. Compensated. The times after fertilization are shown beneath each photograph. Fig. 11 is at metaphase.

PLATE 10

Figs. 1-5. Photographs of eggs of *P. miliaris*, taken from time-lapse films immediately before fixation. $\times 400$. Compensated. The position of the chromosomes, determined by sectioning and staining the individual eggs, is shown diagrammatically beneath each photograph. 1, metaphase; 2, early anaphase; 3, mid anaphase; 4, late anaphase; 5, telophase.

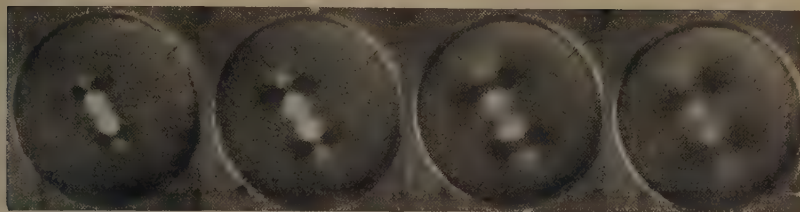


Fig. 1. 56 min.

Fig. 2. 58 min.

Fig. 3. 60 min.

Fig. 4. 62 min.



Fig. 5. 55 min.

Fig. 6. 55 min. 40 sec.

Fig. 7. 56 min. 20 sec.

Fig. 8. 57 min.

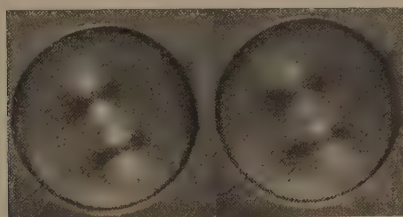


Fig. 9. 58 min.

Fig. 10. 59 min. 30 sec.

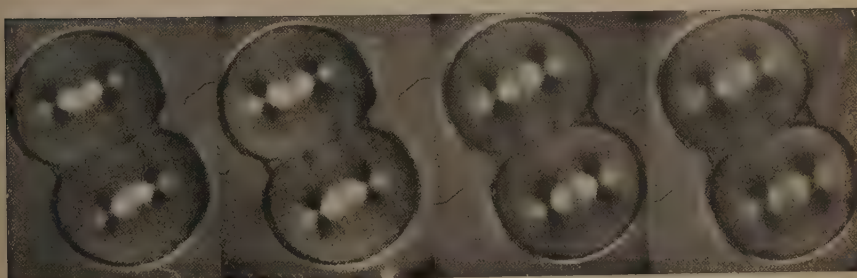


Fig. 11. 104 min.

Fig. 12. 105 min.

Fig. 13. 106 min.

Fig. 14. 107 min. 30 sec.



• | •

Fig. 1.



• || •

Fig. 2.



• || •

Fig. 3.



• | | •

Fig. 4.



• | | •

Fig. 5.

FACTORS DETERMINING THE TEMPERATURE OF TERRESTRIAL ARTHROPODS IN SUNLIGHT

By D. A. PARRY

From the Department of Zoology, University of Cambridge

(Received 21 March 1951)

(With Eight Text-figures)

INTRODUCTION

It has long been realized that the so-called cold-blooded animals are not always cold: in the sun their temperature may rise considerably above that of the surrounding air and even surpass that to which the homoiotherms are regulated. Some measurements have been made of such temperatures (Gunn, 1942), but the experimental difficulties are considerable and can hardly be faced with anything under the size of a large insect. There is, however, another approach to the subject. The temperature of any body in equilibrium with its surroundings, whether animate or inanimate, is that at which the algebraical sum of the heat exchanges across its surface is zero. Furthermore, it will be shown in the third part of this paper that the metabolic heat production of an arthropod on the ground is insignificant compared with the heat received from the sun; and similarly, the amount of this heat lost by evaporation is normally insignificant compared with that lost by radiation and convection. Therefore the temperature of such an animal, like that of an inanimate body, is determined by the balance of radiative, convective, and perhaps conductive, heat exchanges. The factors which influence this balance may be studied with the help of models in a way which would be difficult or impossible with living animals, and by introducing into the model some simplification of shape and colour it is possible to compare the equilibrium temperatures actually recorded with those which would be expected from theoretical considerations.

It must be emphasized that this paper is concerned with the equilibrium temperature of bodies in *direct sunlight*. Such an inquiry has a direct application to those animals, notably grasshoppers and locusts, certain beetles and many arachnids, which habitually expose themselves to the sun. It also has a more general significance inasmuch as the danger of overheating, together with that of desiccation, has most likely played an important part in the evolution of terrestrial arthropods.

FIELD MEASUREMENTS OF EQUILIBRIUM TEMPERATURES

Preliminary measurements

These were made on blackened brass spheres, diameters $\frac{1}{4}$, $\frac{3}{8}$ and $\frac{1}{2}$ in., mounted on Perspex (i.e. low conductivity) feet with the lower surface of the sphere $1\frac{1}{2}$ diameters from the 'ground' (a tufnol sheet flush with rough bare earth). The temperature of

each sphere was measured with a thermocouple made of 0.0048 in. diameter nichrome and constantan wire in which thermal losses were insignificant. The measurements established the following points:

- (a) The temperature of the spheres rose to 40–45° C. on a clear day in June.
- (b) There was a significant difference in equilibrium temperature between the small and medium spheres under the same conditions.
- (c) Slight changes in height above ground had a considerable effect on equilibrium temperature.
- (d) The steep gradient of air temperature within an inch of the ground made it impossible to express measurements as differences in temperature between the body and the immediately surrounding air.

Effect on temperature of height above the ground

(i) *Methods.* To study the effect of changes of height on the temperature of a body it is necessary to make a series of measurements over a period of time during which changes in the sky or the mean wind may occur. Such changes may be at least partially allowed for by expressing the temperatures of the 'experimental' body as differences from those of a 'reference' body similarly exposed in a fixed position. Also for observation close to the ground it is desirable to use a body whose vertical dimension is as small as possible. For these reasons two similar brass disks were used, $\frac{3}{4}$ in. diameter $\times \frac{1}{8}$ in. thick, painted matt black and fitted with type U 2361/20 thermistors (Standard Telephones and Cables Ltd.) connected to a stabilized voltage of about 4 V. Details of a disk, thermistor assembly and the electrical circuit are shown in Fig. 1. The ammeters could either be read directly, or their readings could be recorded by casting a shadow of the needles on a slit beneath which a drum of light-sensitive paper revolved, the light being interrupted at intervals to provide time marks. For calibration the disks were lowered into test-tubes, each containing a thermometer, plugged with cotton-wool and fixed into a large vacuum flask containing a cooling coil. The flask was initially filled with warm water which was cooled a few degrees between each set of readings. A sensitivity of approximately 1 mm./° C. was obtained. The stability of the circuits and the zero settings were checked from time to time by replacing the thermistors by fixed vitreous resistors. It can be shown that neither the heat conducted down the 0.002 in. diameter platinum leads nor the heat generated by the very small current in the thermistors could have a significant effect upon the temperature of the disks.

All the following measurements were made on a sheltered lawn at the Entomological Field Station, Cambridge, during the summer of 1950.

(ii) *Equilibrium temperatures in free air.* At about 2 ft. above the ground the change in temperature with height is very small—less than 1° C. between 18 and 30 in. It is convenient to refer to the disk temperature at such heights as that in 'free air'. Table 1 shows the results of a series of measurements on a disk 30 in. above the lawn on four separate occasions, the temperature given in each case being the mean

difference between disk temperature and shade (screen) temperature, the former of course always being the hotter.

(iii) *Equilibrium temperature close to the ground.* Not only does the disk temperature rise close to the ground but it becomes closely dependent upon the exact height above

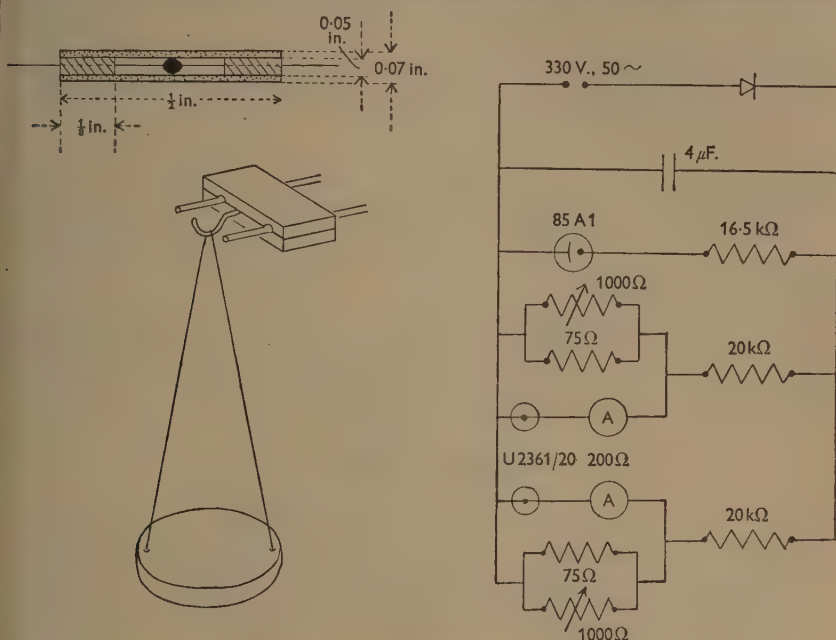


Fig. 1. Details of the disk, thermistor assembly and electrical circuit used in measuring equilibrium temperatures.

Table 1. *Equilibrium temperature of disk 30 in. from ground*

Date (1950)	Time (G.M.T.) and sun's altitude	No. of readings	Air temp. (° C.)	Mean temp. diff. and s.d. (° C.)	Cloud
29 June	09.00-10.30 45-56° alt.	13	24.5	9.0 ± 1.5	Nil
30 June	10.00-15.00 54-61-45° alt.	33	20.5	12.4 ± 1.8	6/10 cumulus
7 July	14.00-16.00 53-36° alt.	20	21.0	9.0 ± 0.9	Hazy, some cumulus
19 Sept.	10.30-12.00 36-39° alt.	22	14.5	20.2 ± 2.8	6/10 cumulus

the surface. A 1 × 1 in. clearing was plucked in the lawn, and measurements were made with the disk just touching the soil, and at various heights up to 1 in. which was approximately the height of the grass. Simultaneous measurements were made of an exactly similar disk 30 in. above the lawn nearby and the results are expressed

(Table 2 and Fig. 2*a*) as differences in temperature between the two, the disk close to the ground always being the hotter.

These measurements (which are typical of several series) show that the disk level

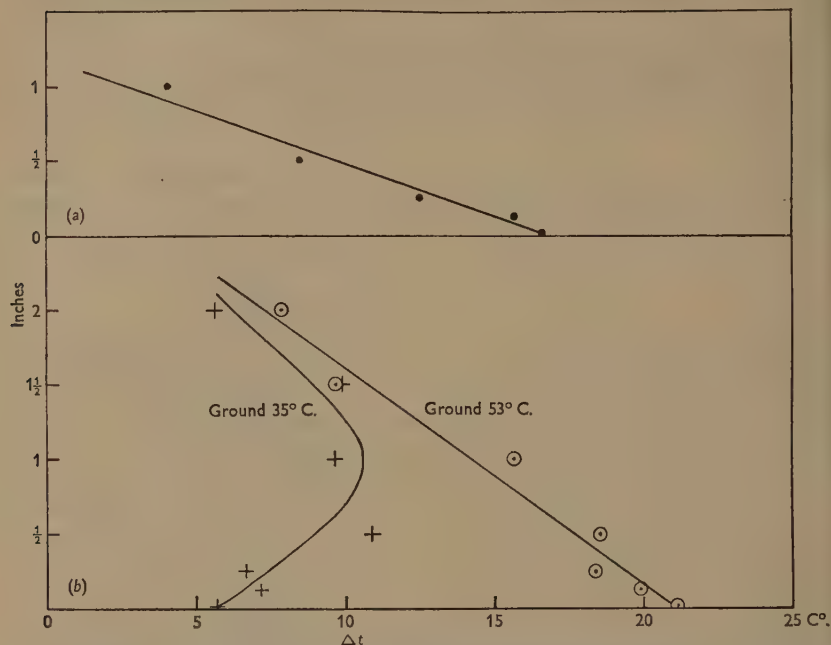


Fig. 2. Variation of equilibrium temperature of a $\frac{3}{8}$ in. disk with height above ground: (a) on a lawn (height of grass c. 1 in.); (b) on an 'artificial lawn' of 2 in. drinking straws on a metal base at two different temperatures of the base.

Table 2. *Effect of height close to the ground on equilibrium temperature*

(29 June 1950, 13.30–14.30 G.M.T., 57–53° altitude, screen temperature 27.0° C., cloud nil.)

Height above soil (in.)	No. of readings	Mean temp. difference (° C.)	Mean temp. reference disk (° C.)
0	6	16.6 ± 1.7	36.9 ± 1.9
$\frac{1}{8}$	3	15.7 ± 0.1	38.1 ± 0.4
$\frac{1}{4}$	4	12.5 ± 0.8	38.0 ± 0.3
$\frac{1}{2}$	3	8.5 ± 0.6	39.4 ± 0.6
1	4	4.1 ± 0.5	39.3 ± 0.1

with the tips of the grass is about 5° C. hotter than the similar disk in free air (30 in.); and that within the grass itself there is a very steep gradient so that the disk touching or almost touching the ground is about 15° C. hotter than the disk in free air. As the latter may be 10° C. or more hotter than the shade temperature, the

disk almost touching the ground may be 50° C. Actually the highest temperature recorded was 55° C.

(iv) *Effect of ground temperature.* Air temperature increases with decreasing height above a warm surface, and this may be expected to cause the rise in equilibrium temperature of the disk shown in Fig. 2*a*. But wind velocity falls with decreasing height, and to estimate the relative effect of the two factors an 'artificial lawn' was constructed, the 'ground' temperature of which could be both measured and controlled. The 'lawn' was made of drinking straws 2 in. high spaced randomly

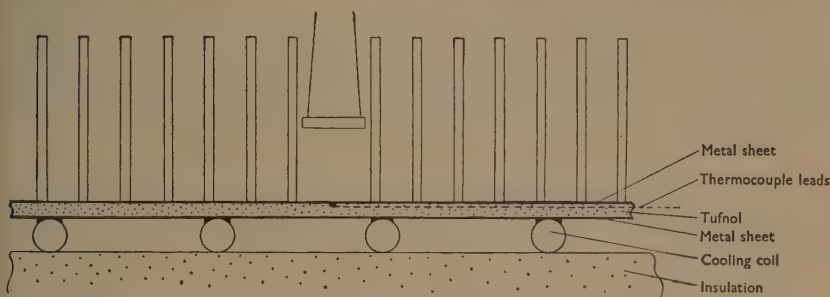


Fig. 3. Details of 'artificial lawn'.

Table 3. *Effect of ground temperature on disk temperature*

(4 Aug. 1950, 12.00–14.00 G.M.T., 54–48° altitude, screen temperature 22° C., cloud 3/10 cumulus.)

Height above 'ground' (in.)	Mean ground temperature 53° C.		Mean ground temperature 35° C.	
	Temp. difference (° C.)	Ref. disk temp. (° C.)	Temp. difference (° C.)	Ref. disk temp. (° C.)
Just clear	21.2	32.6	5.7	32.8
$\frac{1}{8}$	19.9	31.5	7.2	32.8
$\frac{1}{4}$	18.3	31.9	6.7	32.4
$\frac{1}{2}$	18.5	36.9	10.8	33.4
1	15.6	33.2	9.6	33.0
$1\frac{1}{2}$	9.7	33.6	9.8	35.8
2	7.8	34.2	5.6	35.2

about $\frac{1}{2}$ in. apart on a galvanized iron sheet 18 × 18 in., beneath which was a cooling coil (see Fig. 3). The variation of disk temperature with height was found: first, when the 'ground' was uncooled; and secondly, when it was lowered 18° C. Results are shown in Table 3 and Fig. 2*b*. As usual, the temperature of the experimental disk is expressed in terms of the difference from a similar (cooler) disk at a height of 30 in. nearby. The results show the overriding effect of surface temperature upon changes in disk temperature with height: when the ground is cooled the air close to it is also cooled, while that higher up is warmed by the sun-warmed 'vegetation'; and the disk temperature reflects this profile. It is interesting to note the similarity between this profile and that of air temperature among natural

vegetation (Geiger, 1950, p. 282), where a similar effect is produced by the vegetation intercepting the solar radiation and so leaving the underlying ground relatively cool.

Effect of absolute size on equilibrium temperature

It will be shown later that there are good reasons for expecting that the equilibrium temperature of similar bodies will depend on their absolute size. That this is the case was shown by measuring the temperature in free air of three matt black disks $\frac{3}{4} \times \frac{1}{8}$ in., $\frac{3}{8} \times \frac{1}{16}$ in., and $\frac{3}{16} \times \frac{1}{32}$ in. Temperatures were measured with 0.0016 in. nichrome-constantan thermocouples embedded in soft solder in a groove cut along a diameter of the disks. At about 15 cm. from the disk the leads were brazed to thicker wires of the same alloys and the circuit was completed through a galvanometer and melting-ice cold junction. The disks were calibrated in a thermostatically controlled water-bath whose temperature was changed in steps of about 5° C. over the required range. A sensitivity of about 1.5° C./mm. was obtained.

Table 4. *Difference of temperature between similar disks of different size*

(19 Sept. 1950, 10.30–12.00 G.M.T., 36–39° altitude, screen temperature 14.5° C., cloud 7/10 cumulus.)

Temp. group (° C.)	No. of observations	$\frac{3}{4}$ in. disk. Mean temp. (° C.)	$\frac{3}{8}$ in. disk		$\frac{3}{16}$ in. disk	
			Mean temp. (° C.)	<i>t</i> * (° C.)	Mean temp. (° C.)	<i>t</i> * (° C.)
29	1	28.9	25.2	3.7	23.8	5.1
31	3	31.2	27.2	4.0	25.9	5.3
33	4	32.6	27.9	4.7	26.0	6.6
35	7	34.8	29.4	5.4	27.3	7.5
37	1	36.9	31.2	5.7	29.5	7.4
39	5	38.4	32.3	6.1	29.5	8.9
Mean	21	34.2	29.5	4.7	27.3	6.9

* *t* = difference in mean temperature from that of the $\frac{3}{4}$ in. disk.

The results consist of about twenty almost simultaneous observations of the temperature of the three disks. The temperatures of the $\frac{3}{4}$ in. disk have been arranged in two-degree groups and the means of these temperatures have been calculated together with the corresponding means for the $\frac{3}{8}$ and $\frac{3}{16}$ in. disk. These figures are given in Table 4, and they show that the temperature of the disks does decrease with size so that when the temperature of the $\frac{3}{4}$ in. disk ranges between 29 and 39° C., the $\frac{3}{8}$ in. disk is 3.7–6.1° C. cooler and the $\frac{3}{16}$ in. disk is 5.1–8.9° C. cooler. Fig. 4 shows the dependence upon size of the mean temperatures of all the observations on each disk.

Correlation between temperatures of a disk and an insect

Owing to the persistent bad weather during the summer when this work was done very few measurements of living animals were made, nor did an opportunity arise of comparing the temperature of an animal with that of a similarly shaped and coloured model. Comparisons between the temperature of the $\frac{3}{4} \times \frac{1}{8}$ in. matt black

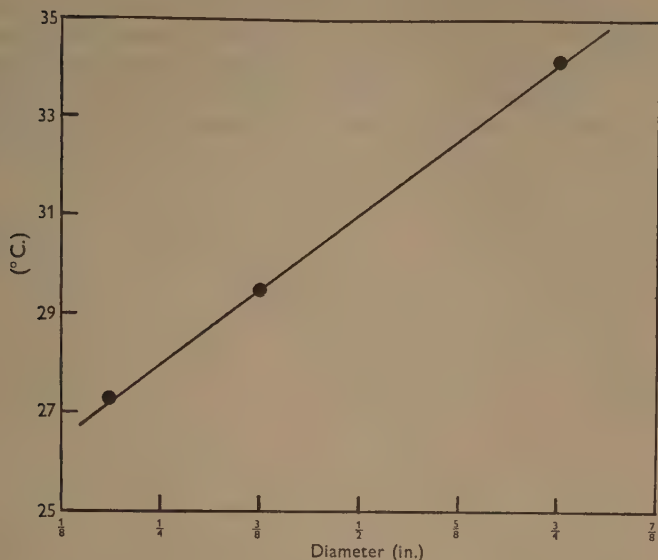


Fig. 4. Effect of size on the equilibrium temperature of a disk in free air.

disk already described, and a living dark phase *Locusta migratoria migratorioides* 2 in. long, were made on two occasions. The locust was orientated broadside to the sun. Owing to the differences in shape between the two bodies, any coincidence between their temperatures must be regarded as fortuitous, but if the same factors are determining the temperature of each a good correlation would be expected between the two. Such a correlation is shown in Fig. 5.

The temperature of the locust was measured by passing an insulated nichrome-constantan thermocouple (0.0048 in. diameter wires) through the anus as far as the base of the abdomen, the insect itself being mounted on a 1 mm. diameter glass rod by thick cellulose enamel paint applied to the ventral surface of the thorax and the feet. The thermocouple sensitivity was 1 mm. = 0.8° C.

A THEORETICAL TREATMENT OF THE TEMPERATURE EQUILIBRIUM OF SMALL ANIMALS IN SUNLIGHT

The factors involved in the heat exchange

(i) *The condition of equilibrium.* Any body is in temperature equilibrium with its surroundings when the net heat exchange across its surface is zero. This therefore is the condition which determines the equilibrium temperature (henceforth called the 'temperature', equilibrium conditions being assumed) of an animal. The types of heat exchange are: net radiation, convection, conduction, metabolism, evaporation.

(ii) *Radiation.* The net radiation load is made up of both solar and long-wave radiation. *Solar radiation* ($0.3\text{--}2.0\mu$ in wave-length with about half the energy in the infra-red) is received directly, diffusely by the sky, and by reflexion from the ground. *Long-wave radiation* (a broad band with a maximum at about 10μ), which all matter produces by virtue of its absolute temperature (5.7×10^{-9} mW./cm.² and

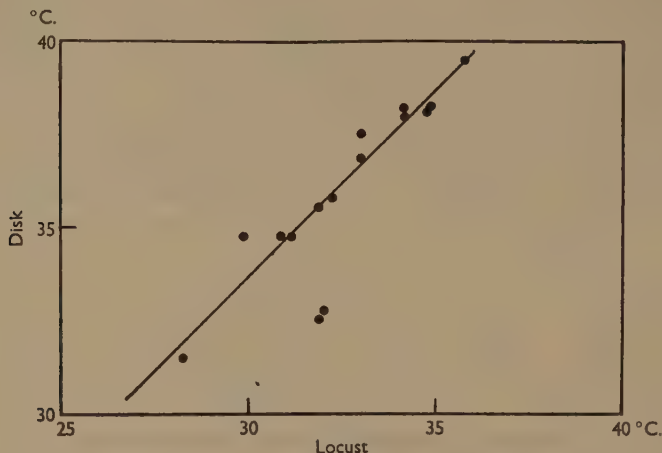


Fig. 5. Correlation between the equilibrium temperature of a locust and a $\frac{3}{4}$ in. disk.

Table 5. *Estimations of the radiation conditions about noon on a clear summer day in southern England*

Direct solar radiation	+*	90 mW./cm. ² normal to sun's rays (Stagg, 1950)
Diffuse solar radiation	+	30 mW./cm. ² on horizontal upward surface (Stagg, 1950)
Reflected solar radiation	+	Reflectivity of grass about 20% (Brunt, 1934)
Atmospheric long-wave radiation	+	35 mW./cm. ² on horizontal upward surface (Robinson, 1950†)
Long-wave loss towards the sky	-	55 mW./cm. ² from a horizontal upward surface (see text)
Long-wave exchange between body and ground	+	10 mW./cm. ² on a horizontal downward surface (see text)

* + indicates radiation received by body; - indicates radiation lost by body.

† I am greatly indebted to Dr Robinson for sending me some additional, unpublished, data which confirm that the figure I have taken is typical of the conditions being considered.

degrees absolute), is both lost by the body and also gained from the surrounding ground and the water vapour and carbon dioxide in the atmosphere. Clearly the values of these various components will vary with meteorological and topographical conditions and the figures given in Table 5 have been chosen as approximate values for the radiation falling on a body above a grass surface within 2 hr. of noon on a summer day with little or no cloud in southern England.

The long-wave radiation from any surface at $30\text{--}50^\circ\text{C.}$ is about 55 mW./cm.^2 ,

but that from a surface facing towards the ground is conveniently set against the radiation from the ground as the net exchange depends more on the difference of temperature between ground and body than upon the actual temperature of either. The figure of 10 mW./cm.^2 given in the table is for a temperature difference of 20° C. , the ground being hotter than the body. This is a fairly high figure, but even so this item of radiation makes a very small contribution to the total balance.

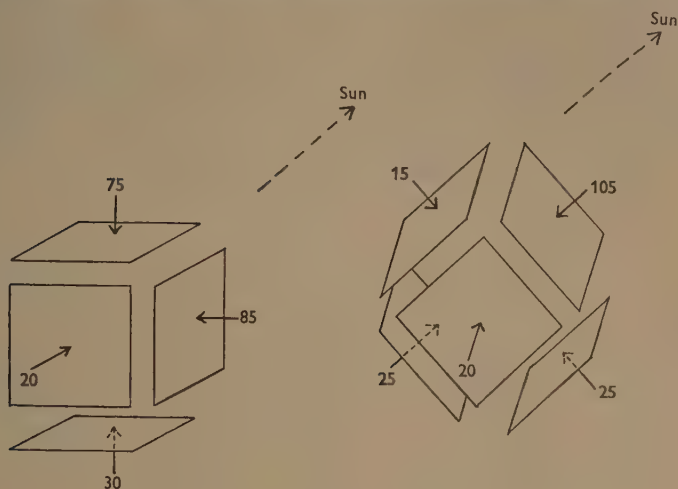


Fig. 6. The net radiation load (mW./cm.^2) on variously orientated black surfaces. Sun's altitude 45° .

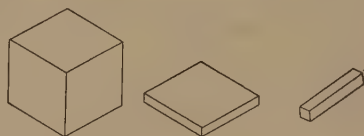
The mean net radiation load per unit surface area of any body will depend, not only upon external conditions, but also upon its own shape, orientation and reflectivity. The effect of these factors can be shown as follows. Fig. 6 gives the net radiation load falling on horizontal, vertical and 45° -inclined plane surfaces under conditions when the figures of Table 5 apply; and these data have been used to construct Table 6 which shows the *mean* net radiation per unit surface area falling on three widely different rectangular shapes orientated horizontally and obliquely, and with a reflectivity to solar radiation of 0 and 50%. The sun's altitude is 45° ; in the horizontal orientation the plane of the sun is perpendicular to one vertical face (a side in the case of the bar), and in the oblique orientation one face (an edge of the plate and an end of the bar) is normal to the sun.

These examples show that the mean radiation load of a body with markedly unequal faces may be varied by a factor of two by re-orientation, the mean of the two extremes being approximately the radiation load of a body with equal faces which is only slightly affected by re-orientation. The examples also show that changes in reflectivity to solar radiation (reflectivity to long-wave radiation being unaffected) produce proportionate changes in the mean radiation load. Data given by Rücker (1933) show the reflectivity of twenty-one insects (mostly beetles) as

varying between 50% (with one example, a white beetle, as high as 74%) and 10%, with a mean of 22%.

(iii) *Convection*. Convective heat loss from a body surrounded by cooler air is due to the heating of the air and its subsequent removal by currents, and is either *natural* or *forced* convection depending on whether the currents are entirely due to density changes consequent upon the heating or whether they are due to an external cause (e.g. wind). Natural convection is likely to be rare in nature, especially under the conditions now being considered when insolation of the ground causes instability in the air and consequent turbulence. The convection coefficient for forced convection (mean heat exchange per unit area per unit difference in temperature between surface and on-coming air) for a body of given shape and orientation with respect

Table 6. *Mean net radiation load for three rectangular shapes*



Orientation	Reflectivity (%)	Cube (mW./cm. ²)	Plate (sides 1 : 8 : 8) (mW./cm. ²)	Bar (sides 1 : 1 : 8) (mW./cm. ²)
Horizontal	0	42	50	50
	50	19	22	23
Oblique	0	35	25	23
	50	15	10	9

to the wind, depends upon the absolute size of the body, the wind speed and the physical properties of the air, according to an expression (McAdams, 1942; Fishenden & Saunders, 1950) of the form:

$$\frac{\text{Convection coefficient} \times \text{linear dimension}}{\text{Air conductivity}} = a \left(\frac{\text{Air speed} \times \text{linear dimension}}{\text{Kinematic viscosity of air}} \right)^b,$$

where a and b are constants over limited ranges of the Reynolds Number (i.e. the right-hand bracket), b being less than one. Thus the convection coefficient varies directly as a fractional power of the wind speed and inversely as a fractional power of the linear dimension of the body. This is illustrated by Fig. 7 (data from McAdams, 1942, p. 221), which shows the effect of wind-speed and size upon convective heat loss from a cylinder (wind blowing across the cylinder). From the definition of the convection coefficient (h) it will be seen that $(100/h)$ (plotted as the ordinate) is the difference in temperature between cylinder and air at which the mean heat loss by convection is 100 mW./cm.² of surface. Fig. 7 thus indicates directly that for a series of cylinders of decreasing size, subject to the same radiation load (and therefore, at equilibrium, losing heat at the same rate per unit area), the equilibrium temperature will decrease with the size of cylinder. It has already been shown experimentally that this is the case for disks of different size (see Fig. 4) and in these

results the apparently linear relation between size and temperature is most likely due to the small range of size considered and not to any fundamental departure from a power law of the type given above.

(iv) *Conduction.* For the purpose of the measurements described earlier, conduction between body and supports was reduced to insignificant proportions. No information has been obtained about the conduction between animals and their substrate, but it may be taken that as a general rule this is small, especially for arthropods whose hard exoskeleton results in poor thermal contact. This is not to deny, however, that an animal might press itself against a yielding surface and make good thermal contact, and under these circumstances direct conduction would cause the animal's temperature to approximate to that of the substrate itself.

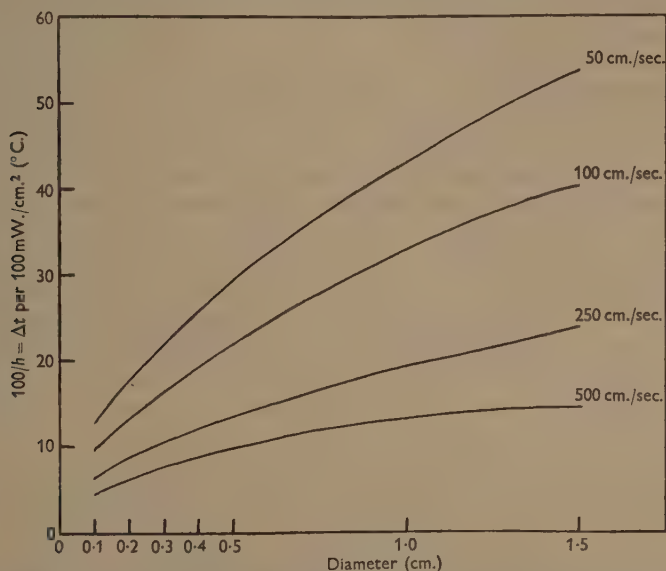


Fig. 7. Effect of size and wind-speed on the temperature difference between a cylinder and the air when the convective heat loss is 100 mW./cm.^2 (data from McAdams, 1942, p. 221).

(v) *Metabolism.* For the present purpose it is necessary to express metabolism in terms of surface area, and whereas many measurements have been made of metabolism (usually rate of oxygen intake) per animal or per unit mass, determinations of area are rare. Butler & Innes (1936) measured the area and oxygen consumption of migratory and solitary *L. migratoria*, and their results varied from 0.0004 to $0.0011 \text{ cm.}^3 \text{ O}_2/\text{cm.}^2/\text{min.}$, which is equivalent to a heat production (at $4.775 \text{ cal./cm.}^3 \text{ O}_2$) of 0.133 – 0.366 mW./cm.^2 . Gunn (1935) found the oxygen consumption of three species of cockroach to lie between 1.1 and $1.6 \text{ mg./cm.}^2/\text{day}$ at 30° C. and the equivalent heat production is 0.197 – 0.286 mW./cm.^2 . A rate of heat production of this order is quite insignificant compared with the radiation load

considered above. Metabolism may rise a hundred times or more during flight, but with this we are not now concerned.

(vi) *Evaporation*. Despite the amount of attention which has been devoted to the humidity relations of terrestrial arthropods, particularly insects, very little information seems to be available concerning the mean rate of evaporation per unit surface area in natural conditions, particularly for species which normally expose themselves to the sun. Observations made in the laboratory are not directly applicable to the conditions now under consideration, because in the laboratory animals are at approximately the same temperature as the air into which they are evaporating, whereas in the sun they may be considerably warmer than the air. Use can, however, be made of the fact (Ramsay, 1935*a*) that evaporation rate is proportional to $(p_o - p_a)$ where p_o is the vapour pressure of air saturated at the temperature of the surface, and p_a is the vapour pressure of the 'free' air some distance away. In laboratory observations when the animal is at approximately the same temperature as the air, $(p_o - p_a)$ is equal to the saturation deficiency; in a typical case of an animal warmed by sunlight to 35° C. in air at 20° C. and 50% relative humidity, $(p_o - p_a)$ is equal to 34 mm. Hg. Thus the cockroach, *Periplaneta americana*, which loses water at 6.0 mg./hr., or about 0.6 mg./hr./cm.², into air at 20° C., 12.0 mm. sat. def. and 10 m./sec. velocity (Ramsay, 1935*b*), would be expected to evaporate at $\frac{3.4}{12} \times 0.6 = 1.7$ mg./hr./cm.² in the circumstances just specified. Similarly, evaporation from three species of cockroach (Gunn, 1935) into still air would be about 0.4 mg./hr./cm.², and that from a freshly engorged tick, *Ixodes ricinus* (Lees, 1947), would be 0.65 mg./hr./cm.². Now an evaporation rate of 1 mg./hr./cm.² involves a heat consumption of 0.67 mW./cm.² so that even if water was lost at four or five times the above rates, the corresponding heat consumption would make a barely significant contribution to the total heat balance of an animal being warmed by the sun.

This conclusion is not, however, to deny that under exceptional circumstances, when an insect's spiracles are fully open (Mellanby, 1934) and perhaps the cuticle has passed its critical temperature (Ramsay, 1935; Wigglesworth, 1945), evaporation might affect the equilibrium temperature at least for a short time. This is borne out by observations on grasshoppers (Gunn, 1942) where it was found that when moist air at 35° C. was replaced by dry ($p_o - p_a = 40$ mm. Hg) the body temperature fell about 4° C., which represents an evaporation rate of about 4.5 mg./hr./cm.² (natural convection coefficient for a cylinder = $\frac{0.42 \Delta t^{1.25}}{d^{0.25}}$ mW./cm.²/° C., where d = diameter (McAdams, 1942, p. 241)).

Heat balance for a body in free air

Data have been given earlier (Table 1) of the equilibrium temperature of a $\frac{3}{4}$ in. disk suspended 30 in. from the ground on a clear summer day. Temperatures between 9 and 20° C. were obtained. In the absence of simultaneous measurements of solar radiation, atmospheric radiation, ground temperature and mean wind speed, it is not possible to draw up an accurate heat balance. However, it is worth while

to make a rough estimate of the expected equilibrium temperature and compare it with the observations.

Radiation. The figures given in Table 5 for the various components of the radiation exchange between a body and its surroundings were chosen to be applicable to the conditions under which the results reported in Table 1 were obtained. The mean net radiation load on a horizontal black disk (diameter=thickness $\times$ 6), calculated from these figures in the way demonstrated for rectangular bodies, is 50 mW./cm.².

Convection. In the absence of published data on the convection coefficient for small disks at low wind-speeds, some original determinations were made (see Appendix), and the results were as follows:

Wind speed (cm./sec.)	50	100	200	400	500
Convection coefficient (mW./cm. ² /° C.)	1.4	2.0	2.7	3.8	4.0

Equilibrium temperature (Δt). This is given, in ° C. above that of the surrounding air, by the expression

$$R = h_c \Delta t,$$

where R =mean net radiation load (mW./cm.²), h_c =convection coefficient (mW./cm.²/° C.). Values of Δt for different wind speeds at $R=50$ mW./cm.² are:

Wind speed (cm./sec.)	50	100	200	400	500
t (° C.)	36	25	19	13	12.5

To associate these wind-speeds with everyday experience it is useful to note that according to the Beaufort scale (*Meteorological Observer's Handbook*, 1942):

Scale no.		Speed at 6 m.* (cm./sec.)
1	Direction shown by smoke drift but not by vane	60-170
2	Felt on face; leaves rustle, wind vane moved	180-330
3	Leaves and small twigs in constant motion; light flag extended	340-520
4	Raises dust and loose paper; moves small branches	530-740

* Speed at 1 m. = $\frac{1}{3}$ $\times$ speed at 6 m. (Best, 1935).

On the occasions when the observations on equilibrium temperature in free air (Table 1) were made there was a gustiness in the wind characteristic of the period about noon on sunny days (Sutton, 1949, p. 15). This made estimations of wind speed particularly difficult, but it most likely was between nos. 2 and 3, or 145-420 cm./sec. at a height of 1 m. This is confirmed by Meteorological Office statistics (*Observatories Year Book*, 1927) which show that in June-August 1925 at Kew Observatory on days when the sun was out at least nine-tenths of the hour ending at noon, the mean wind for that time was 370 cm./sec. at 20 m. which is a little under 300 cm./sec. at 1 m.

At a wind-speed of 150-420 cm./sec. the expected equilibrium temperature according to the above calculation is 22-12° C. which is in good agreement with the observed range of 20-9° C. considering the rough nature of the calculation.

Conditions close to the ground

The principles dealt with above apply equally to animals close to the ground, except that over distances in the order of a millimetre conduction across the layer of air between ground and under surface of body becomes significant, and at the same time air-flow over the under surface is reduced and convective losses consequently fall. However, the actual heat balance and equilibrium temperature in any given case, besides depending on the climatic and morphological factors already discussed, will also depend on so many details of microtopography that only the widest generalization is possible.

As a body approaches a surface warmed by radiation there will be an increase in air temperature and a decrease in wind-velocity while the net radiation load remains the same; hence the equilibrium temperature will rise. The profile of wind and temperature depends on the amount of turbulence in the air: natural surfaces are always more or less rough, causing wind and temperature to change slowly with height except very close to the surface itself. However, most small animals live within the 'roughness', and it is here that conditions are most complex and variable. Wind, and therefore convective loss, is likely to be low, and when the sun can reach the ground to warm it up the temperature profile will be very steep. These conditions prevailed during the observations on the black disk among grass when a temperature change of 12° C. occurred over a vertical distance of 1 in. But with thicker vegetation most of the solar radiation is intercepted before reaching the ground by surfaces which are better cooled than the ground itself; and thus the air temperature is lower and has a maximum somewhere between ground-level and the top of the vegetation, and an animal's temperature will follow this profile as was found in observations with the 'artificial lawn' (Fig. 2*b*).

CONCLUSIONS

The temperature of arthropods in sunlight is determined by the balance between radiative and convective heat exchanges, compared with which the heat associated with metabolism and evaporation is normally insignificant. The radiation load is made up of short-wave radiation received from the sun, directly, diffusely from the sky and by reflexion from the ground; and an exchange of long-wave radiation between the animal and its surroundings, including the atmosphere. The heating due to solar radiation is affected by the colour of the animal, and there may be variations up to 50% in the total radiation load due to this factor alone. The total mean radiation load per unit area will also depend on the shape of the body, and for bodies (such as most arthropods) with markedly unequal surfaces, it may be affected up to about 50% by changes in orientation. The net gain or loss of long-wave radiation is made up of a loss of heat to the atmosphere due to its having an equivalent temperature of approximately 0° C., and a small heat exchange with the ground due to the difference of temperature between it and the animal. Screening the sky by vegetation will reduce the net loss of heat to the atmosphere but will at the same time reduce diffuse solar radiation, so that the resultant effect of changes in the radiation conditions are unlikely to affect the heat balance significantly.

Convective heat loss depends upon the shape of the animal, the wind-speed and the animal's orientation to it, and for a given shape, orientation and wind it also varies inversely as a fractional power of the linear dimension. This means that in any given conditions the smaller an animal is the lower will be the temperature at which convective heat loss balances the radiation load.

It will be evident that the factors determining the temperature of an animal in sunlight, even though they may be reduced to radiation and convection, are very complex and difficult to measure, so that even in the simple case of an animal suspended in free air above the ground a calculation of its temperature will only be very approximate. On the other hand, the insignificance of the 'biological' factors of metabolism and evaporation implies that the temperature of a living animal in a particular situation is likely to be very similar to that of a model of the same approximate size, shape, colour and orientation. Such a model, constructed so that its temperature can easily be measured, may be looked upon as a type of thermometer giving information of considerable biological significance.

When the sun is strong an arthropod, unless it is very small, has at its command temperatures which equal or exceed those of a warm-blooded animal. It remains to be considered to what extent such temperatures are necessary to some animals, and to what extent they are prohibited by the accompanying high rate of evaporation and so represent an important constraint upon the evolution of the habits of terrestrial arthropods. It has already been remarked that, despite the extensive literature on the water relations, particularly of insects, no information seems to be available concerning the rate of evaporation in natural habitats where the animal may be warmed considerably above air temperature, where local humidity, no less than temperature, may be very different from what is reported by the meteorologist, and where sources of water may be available to replenish what is being lost.

SUMMARY

1. A study has been made of the factors determining the temperature of terrestrial arthropods in sunlight.

2. For such animals the most important forms of heat exchange, which determine the equilibrium temperature, are radiation and convection. Compared with these, evaporation and metabolism are insignificant except under unusual circumstances.

3. Convection depends on the size of the body in such a way that the temperature of similar animals in similar circumstances will vary as about the square root of the linear dimension. Other morphological features affecting temperature are shape, orientation and colour.

4. The factors on which such animal temperatures depend are difficult to measure, and it is unlikely that temperatures will ever be accurately deduced from heat-balance considerations. But the insignificance of metabolism and evaporation implies that the temperature of the living animal in a given situation is likely to be very similar to that of an inanimate body of the same approximate size, shape, colour and orientation. If the temperature of such a body can be measured it forms a type of thermometer giving information of direct biological significance.

5. Terrestrial arthropods may be as warm as, or warmer than, the 'warm-blooded' animals, particularly on the ground where conditions change rapidly over small distances so that animal temperatures must be profoundly affected by behaviour.

6. Laboratory data on evaporation at high temperatures do not apply directly to conditions in the field, because an animal warmed by the sun will not necessarily lose water at the same rate as one warmed to the same temperature by warm air at the same relative humidity.

Among several people with whom I have discussed aspects of this work I should particularly like to thank Dr M. Fishenden (convection) and Mr N. E. Rider (atmospheric radiation). I am also indebted to Dr R. H. J. Brown for advice on the construction of the thermistor recorder.

APPENDIX

Natural and forced convection from small disks

Published data on convection is almost entirely limited to wires and cylinders, spheres and large surfaces. The convection coefficients for small disks used in the present paper were original determinations made on a $1 \times \frac{3}{8}$ in. brass disk fitted with a nichrome-constantan thermocouple and a nichrome heating coil of known resistance through which a range of known currents was passed. Natural convection measurements were made in a small sound-proof room almost completely free from draughts; and forced convection measurements were made in an 18×30 in. wind-tunnel, the disk being suspended by the thermocouple wires 9 in. from the nearest side. The wind-tunnel had a straight entry with a 5×5 mm. grid of 1 mm. wire 200 cm. upwind from the disk. Wind-speed was calibrated against propeller r.p.m. by timing smoke puffs.

Calculation. The apparent convection coefficient (h'_c) is given by

$$H = \epsilon A \sigma (T_d^4 - T_a^4) + h'_c A (t_d - t_a), \quad (1)$$

where H = heat input ($I^2 R$), A = area of disk (12 cm.^2 including edges); ϵ = emissivity, taken as 0.2 when unpainted and 0.95 when matt black; σ = Stefan-Boltzmann constant; t_d , T_d , t_a , T_a = temperature in $^{\circ}\text{C.}$ and $^{\circ}\text{Abs.}$ of disk and air.

This ignores heat loss down the thermocouple and heater wires. For a uniform wire one end of which is at temperature t_d and the other at air temperature t_a , the heat loss by convection is given by (Ingersoll, Zobel & Ingersoll, 1948, p. 21):

$$H_w = \frac{1}{2} \pi (t_d - t_a) \sqrt{(k d^3 h_w)},$$

which is of the form

$$H_w = h'_w (t_d - t_a),$$

where k = conductivity of wire, d = diameter of wire, h_w = convection coefficient of wire. Thus the true convection coefficient (h_c) of the disk is given by

$$H = \epsilon A \sigma (T_d^4 - T_a^4) + h_c A (t_d - t_a) + h'_w (t_d - t_a). \quad (2)$$

From (1) and (2),

$$h_c = h'_c - \frac{h'_w}{A}. \quad (3)$$

The heat loss down the heater wires was ignored because of their fineness (48 s.w.g.)

and low heat conductivity. Values of h'_w for the thermocouple wires were calculated as follows, using data on h_w given by McAdams (1942, p. 229):

Wire gauge	h'_w	
	25 cm./sec. wind-speed (mW./° C.)	200 cm./sec. wind-speed (mW./° C.)
40 s.w.g. constantan	0.15	0.23
43 s.w.g. nichrome	0.08	0.12
48 s.w.g. nichrome	0.03	0.05

Thus at

$$25 \text{ cm./sec. } h_c = h'_c - \frac{0.23}{A} \approx h'_c - 0.02,$$

$$200 \text{ cm./sec. } h_c = h'_c - \frac{0.35}{A} \approx h'_c - 0.03.$$

In the present calculations a correction of 0.03 mW./cm.² was applied. It is not possible to apply a similar correction to the measurement of natural convection, but the correction factor is likely to be less than 0.02 mW./cm.².

Results

Natural convection. Seven determinations of the natural convection coefficient were made over the range 22.6–11.7° C. (mean = 16° C.) temperature difference

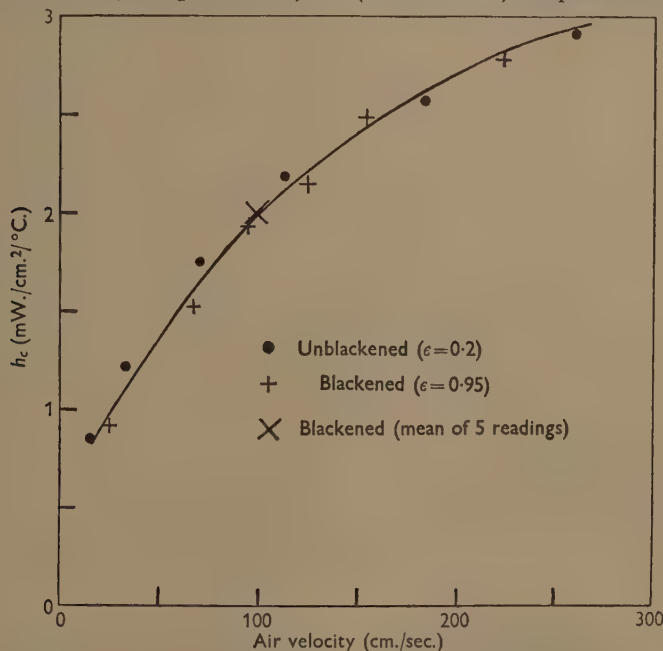


Fig. 8. Convection coefficients of a $1 \times \frac{8}{16}$ in. disk at various wind-speeds.

between the disk and air, the mean film temperature being 28.9°C . The mean coefficient was $0.67 \pm 0.03 \text{ mW./cm.}^2/^{\circ}\text{C}$., the mean log ($Gr \times Pr$) number being 4.38 and the mean log (Nu) number being 0.82. These figures are in good agreement with those given by Fishenden & Saunders (1950, p. 95) for a horizontal plane surface facing upwards.

Forced convection. The forced convection coefficient at different wind-speeds are shown in Fig. 8. The good agreement between the values obtained with a polished and a blackened disk supports the assumed values of the emissivity in the two cases. The range of 2.5×10^2 to 5.0×10^3 in the Reynolds Number is too small to give anything but a very rough indication of the relation between this and the Nu Number. From the data available the relation appears to be

$$Nu = 0.6 \times Re^{0.47}.$$

This is remarkably close to the relation for cylinders for these Reynolds Numbers which is (Fishenden & Saunders, 1950, p. 130)

$$Nu = 0.615 \times Re^{0.436}.$$

REFERENCES

- BEST, A. C. (1935). Transfer of heat and momentum in the lowest layer of the atmosphere. *Geophys. Mem.* **7**, no. 65.
- BRUNT, D. (1934). *Physical and Dynamical Meteorology*. Cambridge University Press.
- BUTLER, C. G. & INNES, J. M. (1936). A comparison of the rate of metabolic activity in the solitary and migratory phases of *Locusta migratoria*. *Proc. Roy. Soc. B*, **119**, 296-304.
- FISHENDEN, M. & SAUNDERS, O. A. (1950). *An Introduction to Heat Transfer*. Oxford: Clarendon Press.
- GEIGER, R. (1950). *Das Klima der bodennahen Luftschicht*, 3rd ed. Braunschweig: Vieweg.
- GUNN, D. L. (1935). The temperature and humidity relations of the cockroach. III. *J. Exp. Biol.* **12**, 185-90.
- GUNN, D. L. (1942). Body temperature in poikilothermic animals. *Biol. Rev.* **17**, 293-314.
- INGERSOLL, L. R., ZOBEL, O. J. & INGERSOLL, A. C. (1948). *Heat Conduction*. New York: McGraw-Hill.
- LEES, A. D. (1947). Transpiration and the structure of the epicuticle in ticks. *J. Exp. Biol.* **23**, 379-410.
- MCADAMS, W. H. (1942). *Heat Transmission*, 2nd ed. New York: McGraw-Hill.
- MELLANBY, K. (1934). The site of loss of water from insects. *Proc. Roy. Soc. B*, **116**, 139-49.
- Meteorological Observer's Handbook* (1942). H.M. Stationery Office.
- Observatories' Year Book* (1927). H.M. Stationery Office.
- RAMSAY, J. A. (1935a). Methods of measuring the evaporation of water from animals. *J. Exp. Biol.* **12**, 355-72.
- RAMSAY, J. A. (1935b). Evaporation of water from the cockroach. *J. Exp. Biol.* **12**, 373-83.
- ROBINSON, G. D. (1950). Notes on the measurement and estimation of atmospheric radiation. 2. *Quart. J. R. Met. Soc.* **76**, 37-51.
- RÜCKER, F. (1933). Die Farben der Insekten und ihre Bedeutung für den Wärmehaushalt. *Pflüg. Arch. ges. Physiol.* **231**, 729-41.
- STAGG, J. M. (1950). Solar radiation at Kew Observatory. *Geophys. Mem.* **11**, no. 86.
- SUTTON, O. G. (1949). *Atmospheric Turbulence*. London: Methuen.
- WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects. *J. Exp. Biol.* **21**, 97-114.

ON THE HORMONAL AND NEURAL CONTROL OF THE RELEASE OF GAMETES IN ASCIDIANS

By D. B. CARLISLE*

Department of Zoology and Comparative Anatomy, University Museum, Oxford

(Received 23 January 1951)

(With One Text-figure)

INTRODUCTION

The homology and function of the neural gland of ascidians have been subjects of controversy for nearly a century since the first description of the organ by Hancock in 1868. The most recent critical discussion of these questions in any language is that by Huus (1937), and the most recent in English is that by Butcher (1930). The various suggestions made can be summarized as follows:

Function:

- Mucus gland (Fol, 1876; Roule, 1884).
- Excretory organ (Julin, 1881*b*).
- Lymphatic organ (Cuénot, 1891).
- Endocrine organ (Butcher, 1930).
- Organ of phagocytic excretion (Pérès, 1943).
- Organ of special sense (Hancock, 1868; Huus, 1937).

Homology:

- Whole pituitary (Julin, 1881*a*).
- Posterior lobe only of pituitary (most recent authors, e.g. Huus, 1937).
- Not pituitary (Pérès, 1943).

The older views have mostly been abandoned or disproved and the two main questions of interest now are:

(1) Is the neural gland of ascidians the homologue of part or of the whole of the pituitary of vertebrates?

(2) If it is an endocrine organ, does it combine some other function with this?

The investigations reported here elucidate these questions to some extent.

NATURE OF THE INVESTIGATIONS

These investigations, on ascidians, are part of a wider study of the mechanisms whereby chordate pituitary hormones are elaborated, and the route by which they elicit their responses. All the experiments reported here are concerned with one such response—the release of gametes—regarded merely as a response, since I am chiefly interested in the route of elicitation.

* This work was performed in the laboratories of the Zoological Station of Naples while holding a Senior Demyship of Magdalen College, Oxford, a Beit Memorial Fellowship for Medical Research, and the Oxford University Naples Scholarship. I wish to thank Prof. A. C. Hardy, Prof. R. Dohrn and Prof. Z. M. Bacq, and Messrs A. E. Needham and W. M. S. Russell.

MATERIALS AND METHODS

The animals used were *Ciona intestinalis* L. and *Phallusia mammilata* (Cuv.), which are both simple ascidians.

The chorionic gonadotrophin used in certain of the experiments was extracted by Mr W. M. S. Russell and myself from human pregnancy urine. It possessed an activity of c. 110 I.U./mg.

The experiments fall into two series—'injection experiments' and 'feeding experiments'—each with their appropriate controls.

(1) I call 'injection experiments' those in which the stimulus to gamete release was provided by means of a single injection of 0.5 or 1.0 mg. (55 or 110 I.U.) of chorionic gonadotrophin dissolved in 1.0 ml. aq. dest.

(2) I call 'feeding experiments' those in which the stimulus to gamete release was provided by feeding to the animal eggs and sperm of its own species. This follows the postulate of Huus (1937) that such a stimulus might be effective (cf. also the demonstration that this is so in sea-urchins by Lindahl & Runnström, 1929).

In all experiments the animals were isolated in individual jars, and the normal controls were not observed to release gametes spontaneously in the aquarium. In 'feeding experiments' the water was changed 2 hr. after feeding so as to remove any remaining eggs and sperm which might have provided a false positive response. The eggs and sperm for feeding were mixed from several individuals, and the mixture was kept for half an hour before pipetting it into the open inhalant siphons of the animals. It contained active sperm, and fertilized and unfertilized eggs at the time when it was added to the jars containing the animals. At present it is unknown which of the three constituents is the active agent. Dosage was large and indefinite.

In some animals in both series of experiments the hearts were removed or nerves sectioned. These various surgical manipulations were performed without anaesthesia or aseptic precautions. In a very few early experiments with *Ciona* sulphanilamide was sprinkled on the water to combat infection; this proved to be unnecessary and was discontinued. The operations were performed by paring away the tunic of *Phallusia* in the appropriate region until the necessary detail was visible; in *Ciona* this was not done since the tunic is thin. Nerves were cut with fine scissors. Blood was removed from the heart with a hypodermic syringe until the flow stopped and then the main mass of the heart and pericardium dissected out.

In other animals the neural gland was destroyed with relatively little damage to the ganglion. This operation was only performed in *Phallusia*. The test over the neural region was pared away and a slit cut through the mantle and pharyngeal wall at one side of the ganglion. The neural gland was then scraped with the tip of a fine scalpel inserted through this slit. After a little practice this operation was quite simple, and the degree of scraping needed to destroy the gland with little damage to the ganglion could be judged. The continued functioning of the ganglion was checked 24 hr. after the operation and at the close of the experiment, by means of the so-called 'crossed reflex' whereby delicate stimulation of one siphon causes closure of the other. According to Prof. Z. M. Bacq (personal communication)

this reflex requires an intact and functioning ganglion. In addition, histological checks were performed after conclusion of the experiments.

RESULTS

The positive response in all the experiments consisted in the release of eggs and/or sperm into the surrounding water and cessation of feeding. In all neural glands which were examined histologically this was accompanied by the discharge of the secretion; in those *Ciona* where the ovaries were examined histologically corpora lutea were observed after ovulation and never before (Carlisle, 1951). The positive response appeared within about 20 hr. at 16° C.

Table of results

Stimulus	Species	Condition of animal	Site of injection	Number	Response
Injection of distilled water	<i>Ciona</i>	Normal	Systemic	11	—
	<i>Phallusia</i>	Normal	Systemic	11	—
Injection of neural gland extract of <i>Ciona</i>	<i>Phallusia</i>	Normal	Systemic	9	6+
Injection of chorionic gonadotrophin	<i>Ciona</i>	Normal	Systemic	11	3—
	<i>Phallusia</i>	Normal	Systemic	11	+
	<i>Ciona</i>	GN	Systemic	3	—
	<i>Phallusia</i>	GN	Systemic	5	—
	<i>Phallusia</i>	GN, unilateral	Systemic	1	+
	<i>Ciona</i>	sn	Systemic	3	+
	<i>Phallusia</i>	sn	Systemic	6	+
	<i>Phallusia</i>	H	Gonad region	7	—
	<i>Phallusia</i>	H	Neural region	8	6+
	<i>Phallusia</i>	H, GN	Neural region	7	2—
	<i>Phallusia</i>	H, sn	Neural region	8	—
	<i>Phallusia</i>	SNG	Systemic	8	+
	<i>Ciona</i>	Normal	...	10	+
Fed with carmine or indian ink	<i>Ciona</i>	Normal	...	5	+
Fed with <i>Ciona</i> eggs and sperm	<i>Phallusia</i>	Normal	...	2	—
Fed with <i>Phallusia</i> eggs and sperm	<i>Ciona</i>	Normal	...	5	—
	<i>Phallusia</i>	Normal	...	5	+
	<i>Phallusia</i>	GN	...	8	—
	<i>Phallusia</i>	sn	...	8	+
	<i>Phallusia</i>	H	...	8	+
	<i>Phallusia</i>	SNG	...	8	—

GN, nerves to the region of the gonads cut near the ganglion.

sn, nerves to the rest of the body cut near the ganglion.

H, heart destroyed and blood removed.

SNG, neural gland destroyed but ganglion left intact.

The positive response consists in the release of gametes and the cessation of feeding.

(1) Experiments involving injection of chorionic gonadotrophin and of other substances

Eleven normal *Ciona* and eleven *Phallusia* all gave positive responses to an injection of chorionic gonadotrophin. The same number of each gave negative responses to an injection of distilled water.

Of nine *Phallusia* which received between them the extract prepared from the

neural complexes of 1000 *Ciona* (by the method of Carlisle, 1950c), six gave positive responses and three negative.

When the nerves running back from the ganglion to the region of the gonads are cut near the ganglion this positive response to gonadotrophin is prevented. Three *Ciona* and five *Phallusia* gave a negative response to gonadotrophin under these conditions. This is not merely due to trauma. The operation does not interfere with feeding or defaecation (cessation of feeding is an invariable part of the positive response); this therefore cannot be a false negative response. Equivalent damage to the neural region and section of all the nerves which run in other directions than towards the gonad does not prevent the positive response; three *Ciona* and six *Phallusia* all gave positive responses when such control operations were performed prior to injection with gonadotrophin. In one *Phallusia* which gave a positive response upon injection of chorionic gonadotrophin after section of the nerves to the region of the gonads, examination showed that the denervation had been only unilateral.

When the heart of *Phallusia* is destroyed and the blood removed the animal continues to live and eventually regenerates these tissues. When an animal which has undergone this operation so recently that regeneration has not begun is injected with chorionic gonadotrophin direct into the region of the gonad, there is no response. Nine specimens were so treated, the injection being made 24 hr. after the operation; of these two died and seven gave a negative response and continued feeding and defaecating. Post-mortem examination showed that they were sexually ripe.

When chorionic gonadotrophin is injected into a similar animal direct into the neural region, the animal gives a positive response. Of eight specimens so injected 24 hr. after the operation six gave positive responses and two negative. When in an animal without a heart the nerves to the region of the gonads are cut, the response to an injection of chorionic gonadotrophin into the neural region is negative. Of eight animals, in which I cut these nerves near the ganglion 24 hr. after destruction of the heart and made the injection into the neural region 2 hr. later, seven gave a negative response and one died. When the other nerves were cut under like conditions, but the nerves to the region of the gonads left intact, the response was positive. Eight animals had the heart destroyed, then after 24 hr. I cut nerves (except those to the region of the gonads) near the ganglion, and after another 2 hr. I injected the animals with chorionic gonadotrophin into the neural region. All gave positive responses,

Eight *Phallusia* had the neural gland destroyed and the ganglion left intact; after 24 hr. each received an injection of chorionic gonadotrophin. All gave positive responses.

(2) *Experiments with feeding*

Five *Ciona* fed with *Ciona* eggs and sperm all gave a positive response. Five *Ciona* fed with *Phallusia* eggs and sperm gave a negative response. Eight *Phallusia* fed with eggs and sperm of *Phallusia* all gave a positive response. Two *Phallusia* fed with eggs and sperm of *Ciona* gave a negative response.

Ten *Ciona* fed with carmine or indian ink particles gave a negative response. A number of these particles was always to be found subsequently in the neural gland.

As in injection experiments it was found that the positive response was prevented by section of the nerves to the region of the gonad (eight *Phallusia*) but not by section of the other nerves (eight *Phallusia*) nor by destruction of the heart and removal of the blood (eight *Phallusia*). Many *Ciona* were killed 2-3 hr. after feeding with eggs and the neural gland examined. Eggs and sperm were always found in the neural gland.

Eight *Phallusia* had the neural gland destroyed and the ganglion left intact, and then after 24 hr. were fed with eggs and sperm of *Phallusia*. All gave a negative response.

DISCUSSION

In the introduction two questions were posed, but before discussing these it might be well to consider the nature of the 'positive response'. Ascidians are highly efficient filter-feeders and filter a large volume of water. (It is estimated that a medium-sized specimen of *Phallusia nigra* filters 173 l. in 24 hr.) One of their difficulties perhaps is to avoid eating their own faeces and gametes. This may be the reason (speaking teleologically) for the heavy mucoid faeces which will not divide into flocculent particles. The more important purpose of avoiding eating their own gametes, which are, of course, floating, is attained by the method of cessation of feeding for long enough for the gametes to be dispersed. The release of gametes in vertebrates is always immediately followed by the discharge of secretion by the anterior pituitary (vide discussion by Burrows, 1945). So also in *Ciona* and *Phallusia* the neural gland discharges its secretion immediately after the release of gametes. Corpora lutea have been observed in the ovaries of *Ciona* after ovulation (Carlisle, 1951).

Is the neural gland homologous with the pituitary? Pérès (1943) believes it is not. He maintains that the various activities interpreted as hormonal by other authors are all due to histamine. This view is inadmissible. The activities concerned are an oxytocic activity (Butcher, 1930; confirmed by Bacq & Florkin, 1935), a vasopressor activity (Bacq & Florkin, 1935) and a chromatophorotrophic activity (Bacq & Florkin, 1935; Abramowitz, 1937). No further evidence has been produced with regard to the first two of these, but even at the time when Pérès wrote, it had been established that histamine has no chromatophorotrophic effect (vide Hogben, 1924). Carlisle (1950b) tested this once more and found that dilute extracts of the eye-stalk of *Leander*, extracts of the neural gland of *Ciona* and of mammalian pituitary posterior lobe (commercial preparation) all expanded the xanthophores of *Leander*, whereas histamine did not. At the same time he found that the chromatophorotrophic principle is confined to the neural gland and is not found even in the adjacent organs and certainly not in the rest of the body, whereas histamine (according to Pérès) is distributed equally over the whole body. The chromatophorotrophic activity at least cannot be due to histamine. Nor can the gonadotrophic activity which was demonstrated to be present in extracts of the neural gland by

Hogg (1937) and confirmed by Carlisle (1950*c*); Carlisle found that the activity was not present in the rest of the body.

The endocrinological evidence, then, suggests that the neural gland is the homologue of the pituitary of vertebrates and confirms the anatomical evidence. Until the discovery by Hogg (1937) of the gonadotrophic activity of the neural complex it was believed that this homology was restricted to the posterior lobe only of the pituitary and that the anterior lobe was not represented in the ascidians (vide Huus, 1937). Hogg's discovery, however, suggested that this was not the case. His view that the neural gland represents the whole pituitary can be supported by several slight pieces of evidence:

(1) The method of secretion of the neural gland is comparable only with that of the *anterior* lobe of selachians and is nowhere found in the posterior lobe (vide Butcher, 1930).

(2) The discharge of secretion by the neural gland after ovulation is strictly comparable with that by the anterior pituitary of vertebrates under like conditions.

(3) A mammalian gonadotrophin will stimulate ascidians to ovulate and discharge sperm.

(4) An extract of the neural gland will stimulate an ascidian to discharge gametes.

(5) The chromatophorotrophic principle of the vertebrate pituitary is believed to derive from the intermediate lobe and therefore to be of hypophysial origin, not infundibular (vide Landgrebe & Waring, 1950). This is likely also to apply to the chromatophorotrophic principle of ascidians.

(6) Van Beneden & Julin (1884) showed that a stomodaeal element entered into the formation of the neural gland which thus had a dual origin, stomodaeal as well as neural. This was confirmed by Metcalf (1895).

The weight of evidence would seem to suggest that the neural gland (including ciliated pit) of Ascidians represents the entire pituitary of Vertebrates, adeno-hypophysis as well as neurohypophysis.

Huus (1937) postulated that the neural gland of ascidians acted as a sense organ to detect the presence of gametes of the animal's own species in the imbibed water. By this means, he suggests, it is ensured that all animals in one neighbourhood discharge their gametes simultaneously, and thus a great measure of cross-fertilization ensues. This postulate is discussed below.

From the 'injection experiments' it appears that gonadotrophin does not act directly on the gonads. Injection of chorionic gonadotrophin or of neural-complex extract direct into a normal animal is followed by the release of gametes whatever the site of injection; the blood carries the hormone to the site of its action. After the heart has been destroyed and the blood removed, then the only site at which injection is effective in stimulating release of gametes is the neural region, which accordingly must be the site of action. Section of the nerves from the ganglion to the region of the gonads prevents the positive response so that the immediate stimulus to the gonads to release gametes is presumably nervous. Destruction of the neural gland, so long as the ganglion is left intact does not interfere with the action of gonadotrophin in stimulating the discharge of gametes.

I conclude that when chorionic gonadotrophin is injected into an ascidian, it acts upon the ganglion. When the ganglion has been stimulated in this way, in its own turn it stimulates the gonads by means of nervous pathways, so that they discharge their gametes. The same is likely to be true of the route of stimulation when a gonadotrophic extract of the neural gland is used instead of chorionic gonadotrophin, though this extract has only been used in the intact *Phallusia*.

When an ascidian is fed with eggs of its own species it releases gametes—Huus's hypothesis is confirmed on this point. The sequence of events following feeding on eggs is the same as that following injection of chorionic gonadotrophin, i.e. the positive response is prevented by section of the nerves to the region of the gonads but not by destruction of the heart and removal of the blood. The stimulus from ingested gametes then must act on the neural region. And for the positive response to follow ingestion of gametes the neural gland must be intact, a condition which is not necessary for the positive response to follow gonadotrophin injections. The stimulus, then, must be through the neural gland and must reach the gonads by nervous, not humoral, pathways from the ganglion. The problem then is how it reaches the neural gland and the ganglion.

Van Weel (1940) has shown that the food stream in ascidians carries particles to the mouth of the ciliated pit. Carlisle (1950a) has observed the same in a transparent tunicate, *Salpa*, and has reported that a proportion of particles always lodges in the ciliated pit, and it is postulated that this organ is the sense organ controlling (via the central nervous system) the rate and mode of feeding. In the course of the present investigations I have observed that a proportion of carmine or Indian ink particles fed to *Ciona* is always to be recovered in the ciliated pit or the neural gland. Pères (1943) would interpret these as in the process of a kind of phagocytic excretion. This cannot be true since:

(1) Carmine and Indian ink are defaecated, and there is no sign of their particles ever being absorbed by the gut.

(2) They appear in the neural gland within a few minutes of first feeding.

(3) Direct observation in *Salpa* shows that there they are carried into the ciliated pit by the food current.

(4) Eggs are similarly found in the neural gland after feeding animals with them; these could not survive digestion and excretion by phagocytes in the form of entire particles. It is certain that the particles found in the neural gland are carried there by the food stream, and that they represent a sample of the ingested particles.

The neural gland and ciliated pit are reported not to be innervated in ascidians (Julin, 1881a; Metcalf, 1895, who records one species with innervation of the ciliated pit, viz. *Boltenia*, where he says that the fibres do not cross the basement membrane. He remarks that no case of innervation had been observed previously; nor have there been any records since). The neural gland contains gonadotrophin; and gonadotrophin stimulates the ganglion as described above. Moreover, the neural gland is needed to mediate between the stimulus provided by the gametes and the ganglion. The obvious hypothesis to be induced from these observations is that the neural gland receives a stimulus from the ingested eggs and in response produces

gonadotrophin. The gonadotrophin passes over to the adjacent ganglion by a non-vascular and non-nervous route and causes it to stimulate the gonads by nervous pathways as discussed above. Such a passage of gonadotrophin to the ganglion may

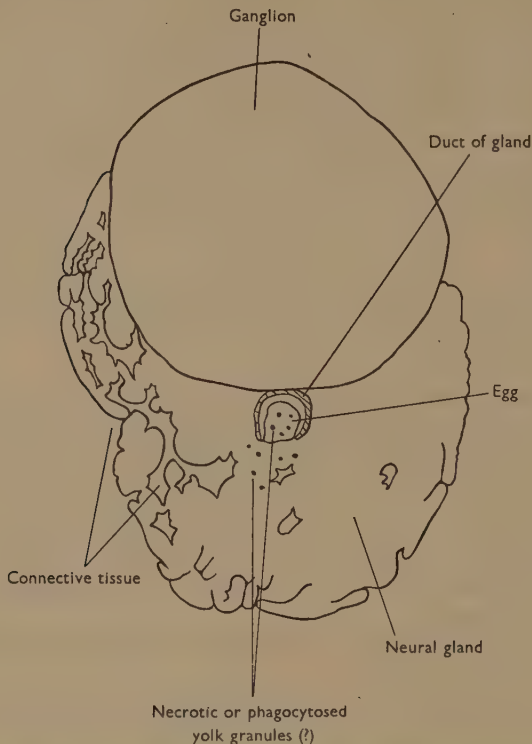


Fig. 1. Transverse section of the cerebral ganglion and neural gland of *Ciona intestinalis* showing an egg in the lumen of the gland. This complex was removed by biopsy from the animal 2½ hr. after feeding with a mixture of eggs and sperm of its own species; it was fixed in mercuric-formal, sectioned at 10 μ and stained with Ehrlich's haematoxylin. Another portion of the lumen of the gland was filled with what appear to be sperm. Other animals of the same batch similarly treated but allowed to retain their neural complex discharged gametes in response to this stimulus. The figure is traced from a photograph.

be compared with the passage of colloid material by non-vascular paths from the anterior pituitary to the hypothalamus in a fish reported by Bretschneider & de Wit (1947).

If this hypothesis is correct then gonadotrophin in ascidians is playing the part of a sensory pathway in a reflex arc:

Stimulus	: Chemical (?) from ingested gametes.
Receptor	: Neural gland.
Afferent pathway	: Hormonal—gonadotrophin.

Central nervous system

Efferent pathway :	Neural.
Effector	: Gonads.
Response	: Release of gametes.

SUMMARY

1. It is argued that the neural gland (+ ciliated pit) of ascidians is homologous with the entire pituitary of vertebrates, adenohypophysis as well as neurohypophysis.
2. *Ciona* and *Phallusia* are shown to respond to an injection of chorionic gonadotrophin by the release of gametes.
3. They respond in the same way to feeding with eggs and sperm of their own species but not to those of other species.
4. This response is prevented in both cases by section of the nerves from the ganglion to the region of the gonads.
5. Destruction of the heart and removal of the blood does not prevent the response to feeding with gametes, nor to injection of gonadotrophin into the neural region; this operation does prevent the reaction if the site of injection is elsewhere.
6. Destruction of the neural gland, leaving the ganglion intact, prevents the response to feeding with gametes, but does not prevent its following an injection of chorionic gonadotrophin.
7. The hypothesis is advanced that the neural gland (+ ciliated pit) is the sense organ involved in this response to feeding, and that it produces gonadotrophin and passes it to the ganglion by a non-vascular route; the ganglion then stimulates by nervous pathways the gonads to release gametes.
8. It is suggested that gonadotrophin is here fulfilling a sensory role in passing information from sense organ to the central nervous system. It may be contrasted with adrenalin which passes instructions from the central nervous system to effectors.
9. *Phallusia* is shown to respond with gamete release to an injection of an extract of the neural complex of *Ciona*.

REFERENCES

- ABRAMOWITZ, A. A. (1937). The comparative physiology of pigmentary responses in the Crustacea. *J. Exp. Zool.* **76**, 407.
- BACQ, Z. M. & FLORKIN, M. (1935). Mise en évidence dans le complexe 'ganglion nerveux—glande neurale' d'une Ascidie (*Ciona intestinalis*) de principes pharmacologiquement analogues à ceux du lobe postérieur de l'hypophyse des vertébrés. *Arch. int. Physiol.* **40**, 442.
- VAN BENEDEN, E. & JULIN, C. (1884). Le système nerveux central des Ascidies adultes et ses rapports avec celui des larves urodèles. *Bull. Acad. Belg. Cl. Sci. Ser. (3)*, **8**, 13.
- BRETSCHNEIDER, L. H. & DE WIT, J. J. D. (1947). *Sexual Endocrinology of Non-mammalian Vertebrates*, p. 70. Amsterdam.
- BURROWS, H. (1945). *Biological Actions of Sex Hormones*, 1st ed. p. 91. London.
- BUTCHER, E. O. (1930). The pituitary in the Ascidians (*Molgula manhattensis*). *J. Exp. Zool.* **57**, 1.
- CARLISLE, D. B. (1950a). Alcune osservazioni sulla meccanica dell'alimentazione della *Salpa*. *Pubbl. Staz. zool. Napoli*, **22**, 146.
- CARLISLE, D. B. (1950b). 'Una localizzazione più esatta del principio cromatoforotropico della regione neurale della *Ciona intestinalis*'. *Pubbl. Staz. zool. Napoli*, **22**, 192.
- CARLISLE, D. B. (1950c). Gonadotrophin from the neural region of ascidians. *Nature, Lond.*, **166**,

- CARLISLE, D. B. (1951). Corpora lutea in an Ascidian, *Ciona intestinalis*. *Quart. J. Micr. Sci.* (in the Press).
- CUÉNOT, L. (1891). Études sur le sang et les glandes lymphatiques dans la série animale. *Arch. Zool. exp. gén.* ser. 2, 9, 13.
- FOL, M. (1876). Ueber die Schleimdrüse oder den Endostyl der Tunicaten. *Morph. Jb.* 1, 222.
- HANCOCK, A. (1868). On the anatomy and physiology of the Tunicata. *J. Linn. Soc. Lond.* 9, 309.
- HOGGEN, L. T. (1924). *The Pigmentary Effector System*, p. 56. Edinburgh.
- HOGGEN, B. M. (1937). Subneural gland of ascidians (*Polycarpa tecta*); and ovarian stimulating action in immature mice. *Proc. Soc. Exp. Biol., N.Y.*, 35, 616.
- HUUS, J. (1937). Tunicata. In Kükenthal, W. & Krumbach, T., *Handbuch der Zoologie*. Also cited in Hanström, B. (1939), *Hormones in Invertebrates*, p. 146. Oxford.
- JULIN, C. (1881a). Recherches sur l'hypophyse et quelques organes qui s'y rattachent, dans les genres *Corella*, *Phallusia* et *Ascidia*. *Arch. Biol. Liège*, 2, 59.
- JULIN, C. (1881b). Recherches sur l'hypophyse et quelques organes qui s'y rattachent chez *Ascidia compressa* et *Phallusia mammilata*. *Arch. Biol. Liège*, 2, 211.
- LANDGREBE, F. W. & WARING, H. (1950). Biological assay of the melanophore expanding hormone from the pituitary. In Emmens, C. W., *Hormone Assay*, p. 141. New York.
- LINDAHL, P. E. & RUNNSTRÖM, J. (1929). Variation und Ökologie von *Psammechinus miliaris*. *Acta zool., Stockh.*, 10, 401.
- METCALF, M. M. (1895). Notes on tunicate morphology. *Anat. Anz.* 11, 329.
- PÉRÈS, J.-M. (1943). Recherches sur le sang et les organes neuraux des Tuniciers. *Ann. Inst. océanogr. Paris*, 21, 229.
- ROUGE, L. (1884). Recherches sur les Ascidies simples des côtes de Provence. *Ann. Mus. Hist. nat. Marseille, Zool.*, 2, 1.
- VAN WEELE, P. V. (1940). Beiträge zur Ernährungsbiologie der Ascidien. *Pubbl. Staz. zool. Napoli*, 18, 50.

THE GROWTH OF BROWN TROUT (*SALMO TRUTTA* LINN.)

IV. THE EFFECT OF FOOD AND TEMPERATURE ON THE SURVIVAL AND GROWTH OF FRY

By MARGARET E. BROWN

Zoological Department, University of Cambridge

(Received 12 March 1951)

(With Nine Text-figure)

Studies of scale development in fishes which live in temperate zones have suggested that variations in the rate of growth of the fish may lead to the development of annuli and circuli on the scales. These variations in growth rate might result from:

- (1) seasonal variations of the physical environment, especially of temperature and illumination;
- (2) variations in the quality and quantity of the food supply; and
- (3) physiological changes within the fish, such as annual cycles of maturation of the gonads and spawning. The second and third factors might well be related ultimately to the first.

The object of the experiments described in this paper was to investigate the problem of the formation of circuli and annuli on the scales by producing trout fry which grew at different rates because of differences in their environment. It is hoped that the results of the examination of these scales will be described later; the present paper is an account of the effect of the conditions used on the growth and survival of the fry.

Gray & Setna (1930) and Bhatia (1931*a, b*) observed that in rainbow trout narrow circuli were produced by a low level of feeding and wide circuli by unlimited food, temperature apparently having no effect. Van Someren (1950) has deduced that in rainbow and brown trout in tropical conditions in Kenya the zones of narrow rings correspond to spawning periods. Cutler (1918) kept plaice and flounders in tanks at different temperatures with much or little food, and showed that the zones on the scales could be correlated with temperature but not with the amount of food. In the present experiments, trout fry were grown at two different temperatures, on three different types of food and at four levels of feeding. The experimental conditions were comparable with those in earlier experiments on the growth of trout fry and 2-year-old trout under controlled environmental conditions (Brown 1946*a, b, c*), but the details of the apparatus and techniques used were different.

APPARATUS AND TECHNIQUE

Trout ova were obtained from the Midland Fishery, Nailsworth, Gloucestershire. They arrived in Cambridge on 23 January 1950, in two batches; one batch consisted of all the eggs of one female trout which had been fertilized with milt from one male

fish, while the other batch consisted of a random sample of the mixed eyed ova used in the hatchery. The ova were kept in hatchery trays at 11° C. until they hatched, and the alevins remained in the same trays until they were ready to feed. Samples of alevins from each set were weighed individually with a torsion balance and measured to the nearest millimetre in order to determine the amount of variation within each set (see Fig. 2 and 3 and Table 1).

Table 1. *Summary of data about ova and alevins*

	Batch of ova from one female and one male trout	Batch of ova from the general hatchery stock
Date of fertilization	16. xii. 49	Not known
Time of hatching: date	30. i. 50-2. ii. 50	26. i. 50-13. ii. 50
as days after fertilization	45-48	Not known
Time of beginning of feeding: date	24. ii. 50	24. ii. 50
as days after fertilization	70	Not known
No. of eyed ova received	1550	1100
No. of freaks	20	26
No. of alevins which survived until beginning to feed	1446	701
No. of alevins which were weighed and measured	216	240
Average weight of alevins in mg.	89.6	109.6
Average length of alevins in mm.	22.4	23.6

Twenty experimental tanks were used; they were aquarium tanks made of glass and angle iron and measured 24 × 12 × 12 in. They were arranged in a row on a tray made of corrugated iron which collected all the overflows and discharged into a sink. A wooden frame 4 ft. above the tanks supported six 2 ft. 20 W. Mazda strip lights (warm white) and was made light-proof above with cardboard. The tanks stood in an alcove so that no light could reach them from behind or at the sides, and a black curtain was hung in front of them. The lights were switched on and off by a Venner time-switch which was set so that the fish received 12 hr. of light and 12 hr. of darkness alternately.

The aquarium tanks were supplied with Cambridge tap water which flowed in from a constant-level tank and overflowed through constant-level siphons so that each tank contained 45 l. of water and water flowed through at the rate of 2 l./hr. Compressed air was bubbled into each aquarium to keep the water well stirred. The water in the tanks was maintained at a constant temperature below that of the tap-water supply by using a cooling system designed by Dr R. H. J. Brown of the Department of Zoology, Cambridge, whose advice and assistance were invaluable.

Cold water was circulated by an electric pump through a series of control-valve units, joined by copper pipes, and returned to a tank which was held a few degrees below the required aquarium temperature by a refrigerator. Each control valve unit was opposite an aquarium tank and had connected to it a spiral of three coils of flexible lead tubing which was suspended in the aquarium (see Fig. 1). The valve unit was a brass block with a straight-through hole and two side openings leading to the aquarium coils. Normally most of the cooling water passed through the

valve block and only a little circulated through the coils but the throughway could be blocked by a sliding plug so that all the cold water was forced to pass through the

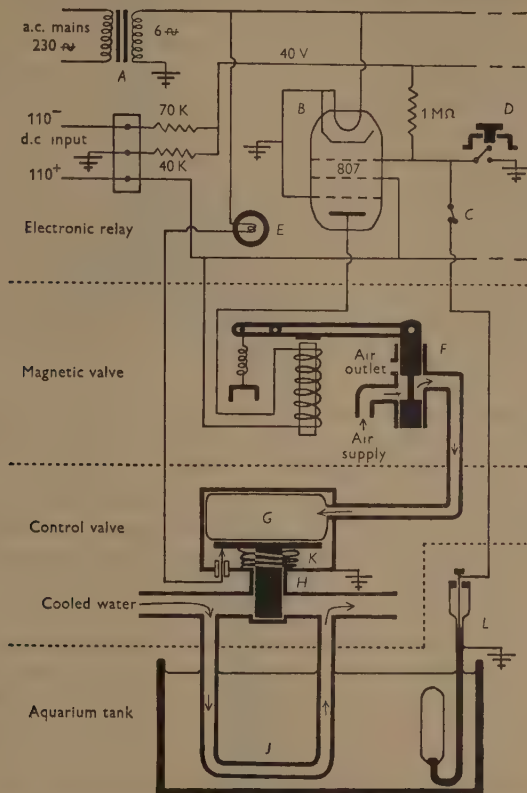


Fig. 1. Diagram of the apparatus used for maintaining twenty aquaria at constant temperatures below room temperature. The parts shown, with the exception of the transformer and d.c. input, are those necessary for one tank only. The diagram is not drawn to scale and shows the valves in the 'on' or 'cooling' positions. *A*, transformer 230 V. to 6 V.; *B*, valve type 807; *C*, switch which is normally closed but is opened before push button test switch *D* is used; *E*, indicator light which is lit when plug *H* is pushed in; *F*, valve controlled by solenoid and spring so that it moves to allow air to pass to *G* when thermostat *L* is closed; *G*, rubber balloon which is inflated when air passes through valve *F* and then pushes in plug *H*, thus forcing cooled water to pass through the cooling coil *J* in the aquarium; *K*, spring which returns plug *H* to the 'off' position when valve *F* is closed, thus allowing the cooled water to flow straight through the control valve unit; *L*, mercury-toluene thermostat immersed in the aquarium.

cooling coils. This plug was moved to the closed position by the inflation of a rubber balloon in a brass chamber and was returned to the open position by a spring.

Each aquarium was fitted with a mercury-toluol thermostat which was connected through an electronic relay to a magnetic valve; this in turn controlled the compressed

air supply to the appropriate control valve in the cooling line, each tank being separately controlled. In the 'off' condition, with the thermostat circuit open, the grid of the 807 valve was held about 40 V. negative from the junction of the 40,000- and 70,000-ohm resistances. No current passed in the anode circuit, and the magnetic valve was unenergized so that the balloon remained deflated and open to the atmosphere. The hole through the control valve was open so that water did not circulate through the cooling coils. When the temperature rose, the thermostat circuit closed and shorted the grid of the 807 valve to earth. About 50 mA. flowed in the anode circuit, energizing the magnetic valve which then connected the balloon to the compressed air supply. The control valve plug was pushed in to block the through hole and thus to cause the cold water to circulate through the cooling coils. When the temperature fell, the system returned to the 'off' condition and the balloon deflated through the magnetic valve. Additional parts of the equipment were: (1) indicator lamps which lit on the relay panel when the movement of the appropriate sliding plugs to the 'on' position was complete, (2) a switching system which allowed the thermostats to be cut out and the correct functioning of the relays to be tested with push buttons.

This system was sufficiently sensitive for the water in the aquarium to be maintained at a constant temperature which varied less than 0.1°C . When there were marked changes in the room temperature and in the temperature of the tap-water supply the rate of circulation of the cooled water and the temperature of the refrigerated tank were adjusted. In really cold weather, the tap water in the constant-level tank was heated by an electric immersion heater controlled by a thermostat so that its temperature did not fall below 13°C . The eight tanks nearest to the refrigerator were maintained at 10.5°C . and the other twelve at 12.5°C .; with occasional breakdowns, these temperatures were maintained throughout the experiments.

The aquaria were stocked with 100 alevins in each on 23 February; 70 of each 100 were taken from the batch of ova derived from a single female and single male trout, and 30 were taken from the mixed batch of ova (see Fig. 3). The alevins began to feed on 24 February, and for 4 days they were all allowed to eat as much as they would of a mixed diet of liver, shrimp meal and live *Tubifex*. Food remains and faeces were siphoned out daily throughout the experiments.

The proper feeding schedule was started on 1 March and was arranged as follows: the eight tanks at 10.5°C . were divided into two sets, each of four aquaria—the fish in one set of four aquaria were fed entirely with minced fresh liver, while those in the other set were given live *Tubifex* worms; the twelve tanks at 12.5°C . were divided into three sets, each of four aquaria—the fish in one set of these were fed with fresh minced liver, those in another set were fed with live *Tubifex*, while those in the third set were given dried shrimp meal mixed to a paste with water. The shrimp meal was too finely dispersed to be suitable for food when the fish grew larger, so from 24 March onwards these fish were given a paste made from 2 parts of shrimp meal to 1 part of Bemax to 1 part of Farex. Even with this mixture the fish did not flourish and their diet was varied on 28 March, 5 and 19 April by giving them live *Daphnia magna*.

The amount of food given to the fish was not measured but when they were fed

they were always given slightly more than they would eat. In each of the five sets of four aquaria, the fish in one aquarium were fed twice daily (once only on Sundays);

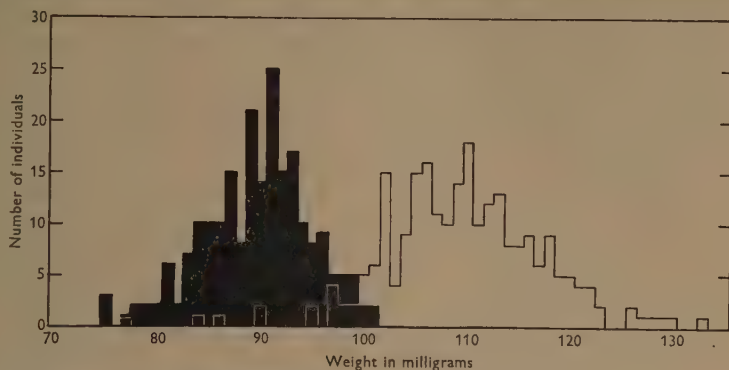


Fig. 2. Histograms to show the distribution of weight among samples of the two batches of alevins. Solid black: alevins derived from a single female trout; white: alevins from the mixed batch of ova.

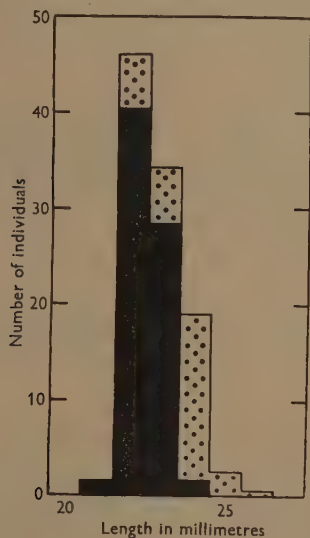


Fig. 3. Histograms to show the average distribution of length among the 100 alevins in each aquarium at the beginning of the experiments. Solid black: alevins descended from a single female trout; dotted: alevins from the mixed batch of ova. Each aquarium was stocked with seventy of the former and thirty of the latter.

in the second they were fed once daily; in the third they were fed three times a week; and in the fourth, once a week only. Thus the ratio of the amount of food available

to the various groups of fish was as 13:7:3:1. By the end of a month it was obvious that the fish which were fed only once a week were receiving less than a maintenance ration, since some had died of starvation. The schedule was therefore altered on 28 March so that these fish were fed twice weekly and those which had been fed three times weekly were thenceforward fed four times weekly. The ratio of food available to the various groups thus became as 13:7:4:2.

The following combinations of environmental conditions were thus available, with 100 trout fry living in each aquarium:

Temperature (°C.)	Type of food	Frequency of feeding per week
10.5	Liver Tubifex	13, 7, 3 later 4, 1 later 2 13, 7, 3 later 4, 1 later 2
12.5	Liver Tubifex Shrimp meal (plus Farex and Bemax after 24 days)	13, 7, 3 later 4, 1 later 2 13, 7, 3 later 4, 1 later 2 13, 7, 3 later 4, 1 later 2

Material for studying the development and growth of the scales was obtained by catching three fish at random from each aquarium each week. These fish were measured, killed and preserved. During the 11 weeks of the experiments, twenty-seven fish were thus removed from each aquarium. The number of fish which died in each tank was also recorded.

At the end of the fifth week, on 2-4 April, all the fish in each aquarium were caught and anaesthetized with 2½% urethane. Their lengths were recorded on a piece of waxed paper attached to a measuring board by pushing their snouts against the perpendicular end of the board and making pricks in the paper at the forks of their tails. The fish were then returned to their tanks and their lengths were read to the nearest millimetre by placing the waxed paper over graph paper illuminated from below.

The experiments were ended on 19 May 1950. All the surviving fish were anaesthetized in urethane, their lengths were recorded on waxed paper and they were preserved in formalin for subsequent examination of their scales.

RESULTS

The survival and growth of fry fed with different types of food

At the beginning of the experiments, the fry would take all three types of food quite readily. They fed only on particles moving or suspended in the water and ignored shrimp meal and bits of liver which sank on to the bottom of the aquaria. The Tubifex worms which fell to the bottom wriggled about but associated themselves into clumps within 2 hr.; these clumps were ignored by the trout and were siphoned out on the following day and used for food again.

It was apparent by the end of 3 weeks that shrimp meal alone was not a suitable diet, so it was reinforced with Bemax and Farex. The fish took this paste readily but remained in such poor condition that on three occasions they were given live

Daphnia which they took eagerly. Towards the end of the experiment the mortality in the groups fed with shrimp meal was very high (see Table 3 and Fig. 4), demonstrating a definite deficiency in the diet.

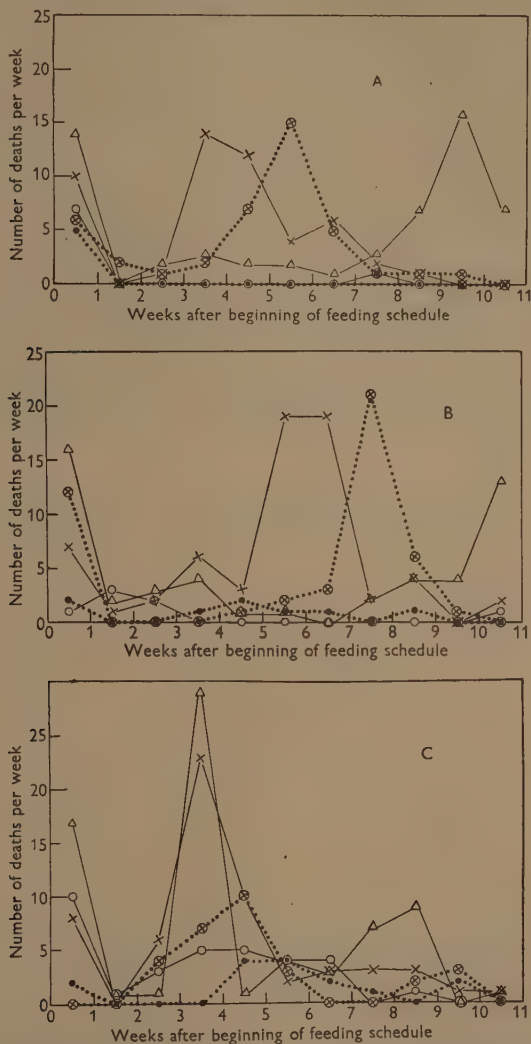


Fig. 4. The number of deaths per week in different groups of trout fry. A, fry fed once per day; B, fry fed three times per week for the first four weeks and four times per week thereafter; C, fry fed once per week for the first 4 weeks and twice per week thereafter. ○ ● fry fed with liver at 12.5 and 10.5° C. respectively; × ⊗ fry fed with Tubifex at 12.5 and 10.5° C. respectively; △ fry fed with shrimp meal at 12.5° C.

An interesting qualitative effect of the shrimp meal diet was that the trout which were fed with it developed orange pigment in the caudal fin and an orange border to the adipose fin. These colours appeared after 3 weeks and were retained till the end of the experiments, whereas the trout fed with liver and *Tubifex* never developed any orange pigment.

The fish which were offered *Tubifex* worms fed eagerly and seemed to be doing well until after the third week, when large fish in these tanks began to die (see Fig. 4A). It was noticed that these fish showed dilation of blood vessels in the skin and bleeding from the tail and fins, but there was no evidence of pathogenic organisms. This phenomenon of the death of well-grown fish continued in these groups of fry fed with *Tubifex* until the end of the experiments, and when the survivors were killed and measured, they all showed this characteristic dilation of cutaneous blood vessels and bleeding from the fins. Either there is some deficiency in a diet consisting of *Tubifex* alone or there is some toxic substance present, and this is accumulated by the trout fry. This deficiency or toxicity becomes lethal as the fish grow larger; this is illustrated by Table 2, in which the mortality rates of fish fed once a day are compared with those of fish fed three or four times a week. As will be described later, the former group grow faster than the latter and their mortality peak occurs 2 weeks earlier than that of the more slowly growing fry.

Table 2. *The number of fry which died per week in groups fed with Tubifex daily and three or four times per week at 10.5 and 12.5° C.*

Each group consisted of 100 fry at the beginning of the experiments.

Temperature (° C.) ...	12.5		10.5	
Frequency of feeding as times per week ...	7	3 (later 4)	7	3 (later 4)
Week 1	10	7	6	12
2	—	1	2	—
3	1	2	1	—
4	14	6	2	—
5	12	3	7	1
6	4	19	15	2
7	6	19	5	3
8	2	2	1	21
9	1	4	1	6
10	1	—	—	1
11	—	2	—	—

Fresh liver was clearly the most satisfactory of the foods used from the point of view of survival of the fry. This is illustrated by Table 3, in which the total numbers of fish dying in the groups fed once and twice daily are given for the 2nd–6th and 7th–11th weeks inclusive. The figures for the first week have not been included, since deaths during that week are less likely to be the result of diet than of injuries sustained in the transfer from the hatchery troughs to the aquaria.

There is no point in comparing the sizes of the survivors in all the tanks at the end of the experiments because of the high mortality and consequent few survivors of the fish fed with *Tubifex* and with shrimp meal. The range of size in the various

Table 3. *The total number of fry which died in groups fed once and twice daily with different diets*

Each group consisted of 100 fry at the beginning of the experiments so the figures represent the number which died out of 200 original fry.

Type of food	Temperature (° C.)	Total no. of fry which died during	
		Weeks 2-6	Weeks 7-11
Liver	10.5	2	7
	12.5	3	3
Tubifex	10.5	54	19
	12.5	50	23
Shrimp meal	12.5	36	57

tanks at the end of 5 weeks, however, provides an interesting comparison, as can be seen from Fig. 5, in which the total range of length, the medians and the upper and lower quartiles are given for fish fed with liver, Tubifex and shrimp meal at 12.5° C.

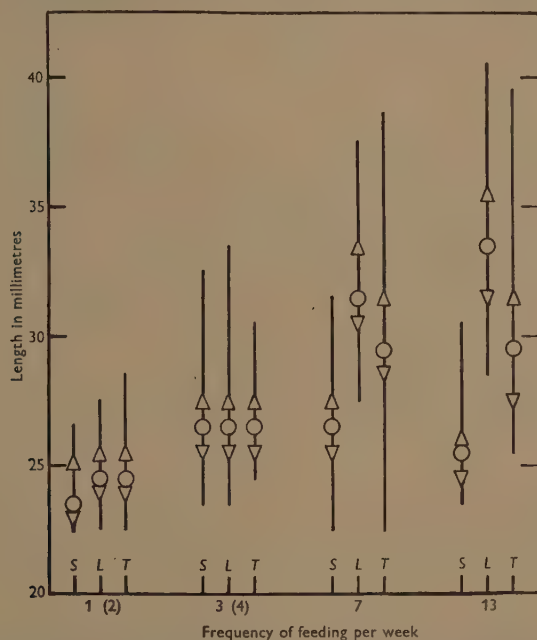


Fig. 5. The distribution of length among groups of trout fry at 12.5° C. after 5 weeks of different diets. The heights of the vertical lines equal the total ranges in length. O median value; Δ upper quartile; ∇ lower quartile; S, diet of shrimp meal; L, diet of liver; T, diet of Tubifex.

The groups are arranged according to the frequency of feeding per week. The fish fed less frequently (1 or 2 and 3 or 4 times per week) show no significant differences

in size which can be related to diet, but those fed once and twice daily with liver attained a significantly greater size than those fed with Tubifex, and the latter were markedly larger than those fed with shrimp meal.

Thus the type of food available for trout fry affects their survival; when plenty of food is available, the rate of growth is determined by the type of food. Of the three diets tested, liver gave markedly better results than Tubifex or shrimp meal.

The effect of frequency of feeding on the survival and growth of trout fry

The numbers of fish which died each week in each tank are shown in Fig. 4. In each of these graphs groups are compared which were fed equally frequently but with different foods and at different temperatures. The groups of fry which were fed twice daily did not differ markedly from those which were fed with the same food once daily, except that there was a higher mortality with shrimp meal as food twice a day than once daily. It was noticeable that the water containing the former group was cloudy with suspended shrimp meal, and this may have caused the higher mortality. The water in the tanks containing fish fed once and twice daily with liver was cloudy with dissolved blood, but this had no adverse effect on the fish. The water containing the fish fed with Tubifex was always very clear.

The figures for fish fed three or four times per week do not differ very much from those fed daily except, as already described, for the delay in the onset of mortality of large fish on the Tubifex diet. The fish fed only once a week apparently did not receive sufficient food, and there was high mortality of small fish during the 4th and 5th weeks. The frequency of feeding was doubled at the end of the 4th week and after that the mortality was low except with shrimp meal—in this tank all the fish except one died before the end of the experiment.

The behaviour of the fish in groups fed at different intervals of time showed interesting differences. Those fed twice daily showed some interest but no great enthusiasm for their food, while those fed once daily were much more excited and appeared to eat more at each feed. The fish fed four times per week or less frequently showed great activity and enthusiasm whenever they were fed, and they ingested so much food that they bulged markedly after each meal and this bulge lasted for more than 24 hr. The fish fed every day never ate sufficient food to distort their streamlined shapes.

Fig. 6 and 7 are histograms showing the frequency distributions of length among the fish fed with liver at 10.5 and 12.5° C. After 5 weeks, and even more markedly after 11 weeks, there was a direct correlation between the frequency of feeding and the size distribution within the groups, the fish fed most frequently having grown largest. At the end of 11 weeks there was no overlap in the size distributions of fish fed once or twice weekly with those fed twice daily and once daily; those fed three or four times weekly had an intermediate size distribution (see also Fig. 9).

Fig. 8 shows that with Tubifex as food there is a similar effect of frequency of feeding on growth, those fed more frequently growing larger than those fed less frequently. There is no real difference between the size distributions of fish fed once and twice daily with Tubifex, and the differences between the groups are less

marked than those between the groups fed with liver; this is the result of the mortality of large fish which began after 3 weeks in the tanks fed once and twice daily.

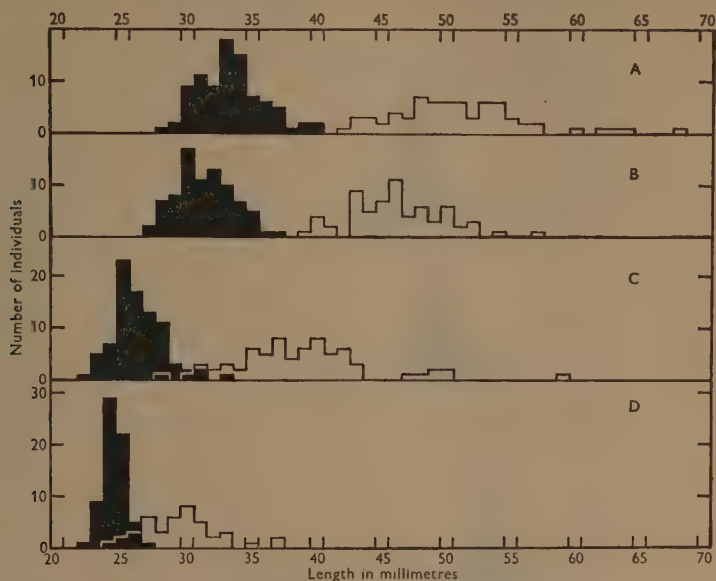


Fig. 6. Histograms to show the distribution of length among groups of trout fry fed with liver at 12.5°C . Solid black: distribution after five weeks; white: distribution after 11 weeks. A, fry fed thirteen times per week; B, fry fed seven times per week; C, fry fed three times per week for the first 4 weeks and four times per week thereafter; D, fry fed once per week for the first 4 weeks and twice per week thereafter.

The fish fed with shrimp meal showed better growth when fed once daily than when fed twice a day (Fig. 5), and there was no significant difference between the sizes of fish fed once daily and three or four times per week. This was probably because the suspension of shrimp meal in the water adversely affected the growth of those fed daily.

Thus the maintenance requirements of trout fry are not satisfied if they are allowed to eat as much as they will only once per week, but when fed twice per week they are able to grow slowly. The amount of growth depends on the frequency with which they are fed, and this was shown most clearly by the fish living on a diet of fresh liver.

The effect of temperature on the survival and growth of trout fry

Two different temperatures only were used in these experiments, and fry were fed with liver and with Tubifex at both these temperatures. There was no apparent difference in behaviour between corresponding groups of fish at the two temperatures, but the mortality curves (see Fig. 4) showed a lag of about 2 weeks between the

maxima for deaths per week at 10.5°C . as compared with 12.5°C . This is also illustrated by the number of deaths of large fish in the tanks fed daily and three or four times weekly with Tubifex (see Table 2), and it is shown in the mortality

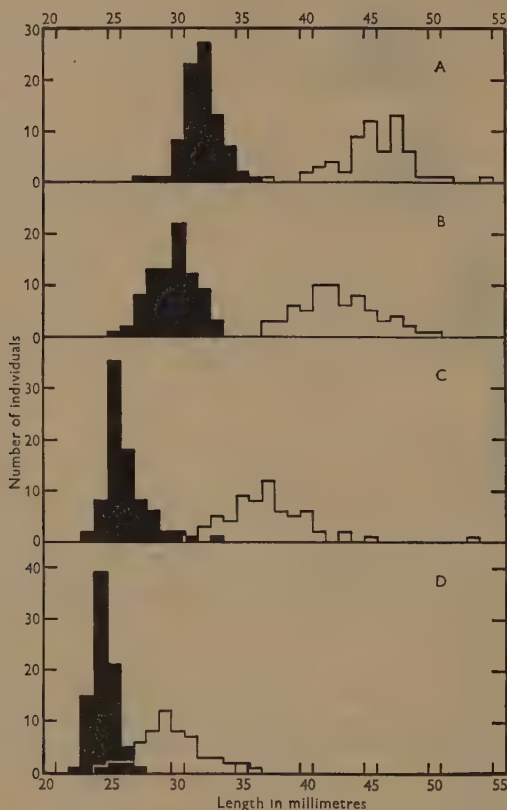


Fig. 7. Histograms to show the distribution of length among groups of trout fry fed with liver at 10.5°C . Conventions the same as for Fig. 6.

curves for fish fed once or twice weekly where there is also a higher mortality in the tanks at the higher temperature (Fig. 4C).

After 5 weeks' growth there were already differences between the size distributions of fish fed once and twice daily at the two temperatures, those feeding at 12.5°C . being larger than those at 10.5°C . (see Fig. 8). The fish fed with liver showed these differences even more markedly after 11 weeks of growth, those at 12.5°C . being significantly larger than the corresponding fish at 10.5°C . (see Fig. 9). Fish fed less often than once daily showed no significant differences in length distribution

after 11 weeks at the two temperatures. Thus with a restricted amount of food there seems to be no difference in the amount of growth at 10.5 and 12.5° C., but with a higher level of feeding the fish at the higher temperature are able to grow

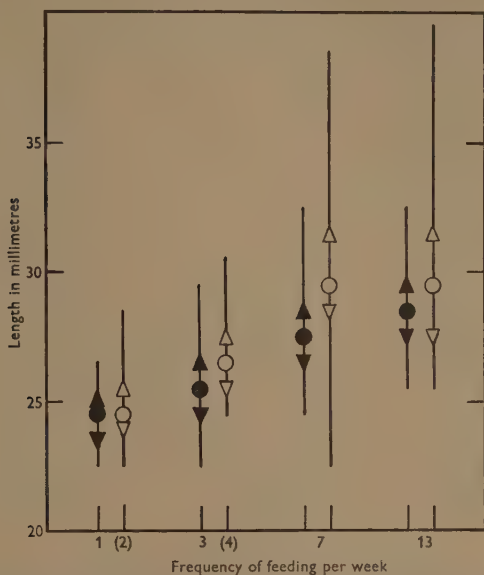


Fig. 8. The distribution of length among groups of trout fry fed for 5 weeks with Tubifex. The heights of the vertical lines equal the total ranges in length. ○ median value; △ upper quartile; ▽ lower quartile; black, fry at 10.5° C.; white, fry at 12.5° C.

faster than those at the lower one. Since the food intake was not measured, this faster growth may have been the result of better appetite or of higher efficiency of conversion of food into fish, or of both.

DISCUSSION

In earlier experiments with trout fry at 11.5° C. (Brown, 1946*a*), liver was the only food used, and the fish were fed twice daily for the first 12 weeks and once daily thereafter. From the point of view of food, therefore, they can be compared with the fish which were fed most frequently with liver in the present experiments. In other experiments with 2-year-old trout (Brown, 1946*b, c*) the effect of level of feeding on growth was investigated at 11.5° C., and appetite and maintenance requirements were measured at five other constant temperatures. In all these experiments, the growth of individual fish was very much affected by their positions in the size hierarchies. The effect of size hierarchies was also apparent in the present experiments, since the disparity in size between the smallest and largest fish increased as the fish grew larger.

McCay, Dilley & Crowell (1929), using *Salvelinus fontinalis*, investigated diets of purified protein, carbohydrates and fat and found that with less than 10% protein the fish did not grow and died within 6 months; with a higher level of protein, the fish grew but died within 3 months. They concluded that there was a store of some essential substance in the trout body which was destroyed during growth and could

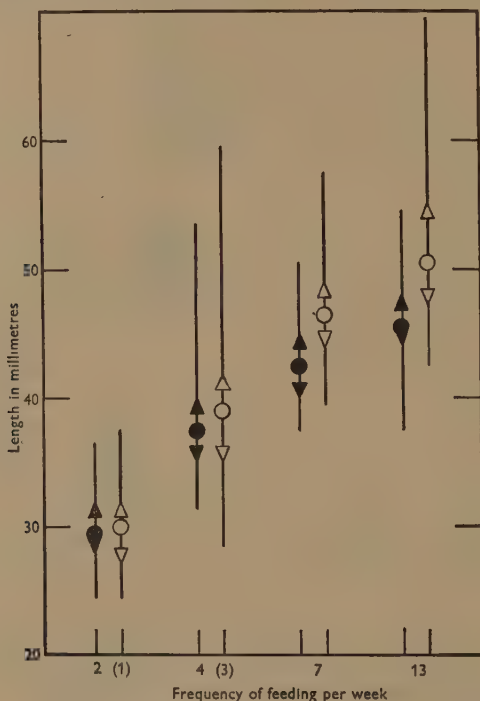


Fig. 9. The distribution of length among groups of trout fry fed for 11 weeks with liver. The heights of the vertical lines equal the total ranges of length. ○ median value; △ upper quartile; ▽ lower quartile; black, fry at 10.5° C.; white, fry at 12.5° C.

not be replaced from the purified diets. This same explanation could account for the mortality of large fry found in the present experiments with *Salmo trutta* using a diet of Tubifex. McCay *et al.* do not refer to any characteristic symptoms being associated with the death of their fish as was bleeding from the fins and dilation of cutaneous blood vessels with trout fry dying on the Tubifex diet. The deaths on this diet could be explained as the result of accumulation of toxins from the food instead of as the result of depletions of essential substances.

McCay *et al.* found that brook trout grew and survived normally when raw liver was added to the purified diets. They also discovered that diets of dried buttermilk, dried skim milk, gluten and pea nut meal could only be utilized for growth when

raw liver was added. Coleman (1930) compared raw liver, salmon-egg meal, shrimp meal, sardine meal, lactein, kelp and alfalfa as food for trout fry, and noted that liver was the most satisfactory and that all the others required to be supplemented with liver if good growth was desired. McCay & Tunison (1937) also found that raw meats were more efficient foods than dried feeds, and Tunison (1940) observed that the higher the amount of protein in the food, the better the growth and the lower the mortality. Hewitt (1943) also remarked that the dried foods used in trout hatcheries lacked some essential dietary constituent which is present in liver. There is thus plenty of evidence that liver contains some substance essential for trout growth and absent from such foods as dried shrimp meal and dried milk. The high mortality and poor growth found in the present experiments with shrimp meal, even when supplemented with Bemax and Farex, and the excellent growth and low mortality of the fry fed with liver are therefore in full agreement with the results obtained by other workers.

Myers (1946) stressed the importance of the first year's growth for the subsequent production of large trout, and stated that natural foods are better than unnatural ones and that zooplankton and dipterous larvae are the best food of all. The present experiments showed that not all species of aquatic animals are adequate trout foods even when alive, since the diet of *Tubifex* led to high mortality and was not nearly as satisfactory as the 'unnatural' food liver. The fish fed with shrimp meal were given live *Daphnia* on three occasions, and their liveliness was much increased by this addition of 'natural' food. Some twenty alevins, not required for experiments, were kept in a separate aquarium and were fed almost entirely with planktonic Crustacea. These fry remained healthy and grew faster than any of the experimental fry fed with shrimp meal. Had it been possible to obtain a sufficiently large and regular supply, planktonic Crustacea would have been used instead of shrimp meal in the present experiments.

Wild stocks of trout show considerable differences in coloration according to their locality, and it has been suggested that these differences are associated with differences in feeding habits. Orange fins and red spots appear to be characteristic of trout which feed largely on Crustacea, and where these organisms are not normal constituents of the diet, red and orange pigments are not conspicuous. Steven (1948) identified and estimated the amounts of various carotenoids in different parts of the body of brown trout. He showed that in aquaria fish fed with horseflesh and earthworms lost 90% of their carotenoids in 9 months, whereas those fed with natural foods, mainly Entomostraca, retained their wild coloration. McCay (1937) noted that additions of salmon ova, goldfish, *Daphnia* or carrots to their diet enabled trout to retain their natural coloration, and Steven (1948) found that fish fed on salmon ova were more pink than normal wild fish. In the present experiments, orange coloration was developed only in those fry fed generally on shrimp meal and three times with *Daphnia*, while the extra alevins fed mainly with planktonic Crustacea, also developed much orange pigmentation. Thus these experiments give further support to the conclusion that the pigments of the lipophores of trout are derived solely from their food. Steven (1948) deduced that trout are unable to

convert one type of carotenoid into another, and the trout fed with fresh liver in the present experiments were not able to produce astacene and lutein in visible quantities in their skins. Thus, the type of food eaten can produce differences in the coloration of the fish as well as in their survival and growth rate.

In earlier experiments (Brown, 1946*a, b, c*) it was found that trout allowed to eat as much as they would had definite appetites and would not eat more than a certain definite quantity of food on each occasion of feeding. Moore (1941) observed the same phenomenon with individuals of *Apomotis*, *Helioperca* and *Perca flavescens*. In the present experiments, the food was not weighed, but the fish were given more than they would eat on every occasion when they were fed. The frequency of feeding ratio was as 13:7:3 (later 4):1 (later 2). As the fish grew larger they consumed more food, though it probably represented less in proportion to their body weights. The differences in behaviour of the different sets of fish was such that those fed less frequently probably consumed relatively more than the others on the occasions when they were fed because they stuffed themselves to such an extent that they bulged and all rested on the bottom of their tanks for 24 hr. afterwards. Those fed twice daily showed less enthusiasm and probably consumed relatively less at each feed than those fed once daily. Thus the real ratio of amount of food consumed per week per unit body weight was probably more like 4:3:2:1 than 13:7:3 (later 4):1 (later 2).

The food consumed by a fish must be used for maintenance of its tissues as well as for growth. The fish which were fed only once per week did not receive sufficient for maintenance, since they were becoming thin and dying 4 weeks after beginning to feed. When their frequency of feeding was doubled this led to decreased mortality and improved condition. Thus, trout fry allowed to eat as much liver, Tubifex or shrimp meal as they will twice per week obtain more than their maintenance requirements. The figures for the fish fed with liver show a clear correlation between frequency of feeding and growth rate, those fed twice daily growing faster than those fed once a day, the latter better than those fed three or four times weekly and those fed once or twice weekly growing least rapidly. Two-year-old fish grow more efficiently if the amount of food given to them is restricted (Brown, 1946*b*), and they may reach almost the same size as comparable fish allowed to eat as much as they would. This phenomenon was not displayed by the trout fry. Myers (1946) states that trout fry do not hunt for their food for some time after they begin to feed, so that they must rely on food coming within their range of vision; they will swim after it and take it. It is not known how much they could consume in one day, and it is possible that they might obtain more than is possible with two feeds daily of minced liver. Thus, even the most frequently fed fish in the present experiments may have been underfed compared with wild trout fry in an environment rich in food.

Mottram (1936) reported experiments with trout fry which gave very different results from those described here. He used plankton as food and divided his fish into four batches, each consisting of twenty-five brown trout fry. One batch was fed daily, another twice a week, the third batch once a fortnight, while the fourth batch

was not fed at all. Mottram states that the fry which were fed twice a week grew as well as those which were fed daily, while those which were fed only once a fortnight remained active and healthy but did not grow as fast as the others. The difference between these results and those described in this paper could be explained if Mottram's fish were given so much live plankton at each feed that those which were fed twice a week had sufficient for them to eat the same amount every day as those which were fed daily, the latter being provided with considerable excess of food. Those which were fed once a fortnight would have received enough food for them to eat well for at least several days. Eight of the twenty-five starved fry died during the first four weeks. The temperature of the water is not recorded.

The marked differences in growth rate between trout fry under natural conditions in different localities have been ascribed to many causes, including differences in the quantity and quality of the food. The present experiments showed that differences in type of food can have marked effects on the survival, growth and coloration of trout fry and also that the frequency of feeding is an important factor, since it was only among the groups fed once and twice daily that significant differences in size could be correlated with differences in diet. This implies that it is only when food supplies are abundant that differences in quality will result in differences in the rate of growth of trout fry. With restricted quantities, the quality of the food should have no effect on the growth rate. With liver, the most successful food used in these experiments, the rate of growth was proportional to the number of feeds per week, while with shrimp meal there was no difference between the growth rates of fish fed three or four times weekly and those fed daily.

Two temperatures only (10.5 and 12.5°C.) were used in the present experiments, but the fry which were fed with liver and those which were fed with *Tubifex* showed that this difference of 2°C. was sufficient for there to be differences in survival and growth of comparable fish. When fed only once per week, fish at 10.5°C. survived better than those at 12.5°C. , indicating that the maintenance requirement must be less at the lower temperature. Fish which were fed once or twice daily grew faster at the higher temperature, but there were no significant differences between those with more restricted rations at the two temperatures.

For 2-year-old trout at constant temperatures, specific growth rates were high between 7 and 9°C. and between 16 and 19°C. , and low above, between and below these temperature ranges. Thus the two temperatures used in the present experiments fall within a minimum growth-rate range for older trout. No data are available for trout fry growing under comparable conditions below 10.5 or above 12.5°C. , so it is not possible to demonstrate whether they have the same growth rate-temperature relationship as the 2-year-old trout.

SUMMARY

Groups of 100 trout fry were grown in identical aquarium tanks at constant temperatures, with 12 hr. of illumination per day, constant rate of water flow, aeration and composition of the water. Two different temperatures, three different types of

food and four levels of feeding were investigated. Individual lengths were recorded for the first 11 weeks after the beginning of feeding.

The trout fry took live *Tubifex* worms eagerly and grew well up to a certain size, when they began to develop bleeding from the fins and dilation of cutaneous blood vessels and to die. It is suggested that *Tubifex* either lacks some chemical substance essential for trout survival or contains some substance which is accumulative poison.

The trout fry which were fed with shrimp meal lost condition and showed poor survival and growth, suggesting that their diet lacked some necessary constituent. These fish developed orange pigment in their caudal and adipose fins; such orange pigment was not developed by fry fed with *Tubifex* or with liver.

The trout fry which were fed with liver had the lowest mortality and showed the best growth, supporting the view that liver contains all the substances necessary for trout growth.

Fry fed twice daily with liver showed less enthusiasm for their food than those fed once daily but the former grew slightly faster.

Fry fed three or four times per week with liver gorged themselves whenever they were fed but grew at lower rates than those fed daily.

Trout fry allowed to eat as much liver as they would once per week gorged themselves, but obtained less than their maintenance requirements and began to die of starvation after 4 weeks at 12.5° C. They were able to grow slowly when fed twice per week.

Fry fed once or twice daily with liver grew larger than those thus fed with *Tubifex*, and the latter grew larger than fry fed daily with shrimpmeal. There were no significant differences in size which could be correlated with diet among fish fed less often than once daily.

Trout fry fed daily at 10.5° C. grew more slowly than those fed daily at 12.5° C., but those fed less frequently showed no differences in growth rate which could be associated with temperature. Those fed with *Tubifex* at 10.5° C. showed a delay of 2 weeks in the onset of high mortality compared with those at 12.5° C.

I wish to thank Dr R. H. J. Brown for his advice and assistance in the construction and maintenance of the temperature-control apparatus and Miss C. M. Sullivan for her kindness in looking after and feeding the fish on occasions when I was away from Cambridge.

Since this paper was written the aquarium temperature control system has been redesigned by Dr R. H. J. Brown to work over a wider temperature range. Individual tanks can be controlled at any temperature between 4 and 35° C. The rubber balloons which were liable to develop leaks are replaced by reinforced rubber diaphragms.

REFERENCES

- BHATIA, D. (1931a). On the production of annual zones in the scales of the rainbow trout (*Salmo irideus*). I. *J. Exp. Zool.* 59, 45-59.
BHATIA, D. (1931b). A critical study of the scales of two specimens of starved and excessively fed trout, *Salmo irideus*. *J. Cons. int. Explor. Mer.* 6, 266-72.

- BROWN, M. E. (1946a). The growth of brown trout (*Salmo trutta* Linn.). I. Factors influencing the growth of trout fry. *J. Exp. Biol.* **22**, 118-29.
- BROWN, M. E. (1946b). The growth of brown trout (*Salmo trutta* Linn.). II. The growth of two-year-old trout at a constant temperature of 11.5° C. *J. Exp. Biol.* **22**, 130-43.
- BROWN, M. E. (1946c). The growth of brown trout (*Salmo trutta* Linn.). III. The effect of temperature on the growth of two-year-old trout. *J. Exp. Biol.* **22**, 145-55.
- COLEMAN, G. E. (1930). Results of feeding experiments with trout fry. *Calif. Fish Game*, **16**, 1.
- CUTLER, D. W. (1918). A preliminary account of the production of annual rings in the scales of plaice and flounders. *J. Mar. Biol. Ass. U.K.* **11**, 470-96.
- GRAY, J. & SETNA, S. B. (1930). The growth of fish. IV. The effect of food supply on the scales of *Salmo irideus*. *J. Exp. Biol.* **8**, 55-62.
- HEWITT, E. R. (1943). Trout growth in America. *Salm. Trout Mag.* **108**, 112-15.
- MCCAY, C. M. (1937). The biochemistry of fish. *Ann. Rev. Biochem.* **6**, 445-68.
- MCCAY, C. M., DILLEY, B. & CROWELL, M. (1929). Growth rates of brook trout reared upon purified rations, upon dry skim milk diets, and upon feed combinations of cereal grains. *J. Nutrit.* **1**, 233-46.
- MCCAY, C. M. & TUNISON, A. V. (1937). Report of experimental work for the year 1936. *Rep. Cortland Hatchery*, no. 5.
- MOORE, W. G. (1941). Studies on the feeding habits of fishes. *Ecology*, **22**, 91-6.
- MOTTRAM, J. C. (1936). Experiments on the starvation of trout fry. *Salm. Trout Mag.* **84**, 264-7.
- MYERS, C. (1946). Trout foods. Some observations on trout growth during their first twelve months. *Salm. Trout Mag.* **118**, 212-18.
- STEVEN, D. M. (1948). Studies on animal carotenoids. I. Carotenoids of the brown trout (*Salmo trutta* Linn.). *J. Exp. Biol.* **25**, 369-87.
- TUNISON, A. V. (1940). The protein requirement of trout. *Progr. Fish. Cult.* **50**, 29.
- VAN SOMEREN, V. D. (1950). The 'winter check' on trout scales in East Africa. *Nature, Lond.*, **165**, 473-4.

BODY SIZE IN RELATION TO OXYGEN CONSUMPTION AND PLEOPOD BEAT IN *LIGIA OCEANICA* L.

By C. ELLENBY

Department of Zoology, King's College, Newcastle-upon-Tyne, University of Durham

(Received 19 March 1951)

(With Seven Text-figures)

INTRODUCTION

The experiments on which the present paper is based were completed in 1938 at University College, London, but for a variety of reasons it has been impossible to prepare the results for publication until now. I had just completed an investigation of the oxygen consumption of prepupae of *Drosophila melanogaster* and had demonstrated the inadequacy of 'body-weight' as a standard of comparison; in fact, the rate of oxygen consumption was shown to be proportional to the surface area (Ellenby, 1937, 1945*a*). It seemed desirable to examine this relationship in other poikilothermal animals; there are numerous cases in which workers have commented on a fall in metabolic rate with increasing body size, but extremely few in which the relationship has been investigated. Clearly, if it is general for the metabolic rate of cold-blooded animals to vary with the size of the animal, it is important for an understanding of metabolic processes that the relationship should be investigated; it might also be of importance for an understanding of aspects of mammalian physiology. Moreover, if the phenomenon were general, control of the 'size factor', rarely considered, would be a first demand in work in which the metabolic rates of different animals are compared. It is for these reasons that the present work was originally undertaken; despite the lapse of time and in spite of the excellent work in this field of Weymouth and his school (Weymouth, Crismon, Hall, Belding & Field, 1944), it is felt that these arguments have lost little of their force.

Ligia oceanica is a very convenient animal for an investigation of this sort. It has a simple shape, can be kept relatively easily in the laboratory, and, for an isopod, has a large size range. The movement of its pleopods has been studied by a number of workers (Nicholls, 1931; Numanhoi, 1933, 1937; Fox & Johnson, 1934; Johnson, 1936), but in no case has the effect of size been investigated. As it was noticed that the rate of beat varied with the size of the animal, it was thought that such an investigation would be of interest in relation to the distinction drawn by Fox (1936) between 'non-locomotory' and 'activity' metabolism of animals from northern and temperate waters.

MATERIALS AND METHODS

The specimens were obtained from Drake's Island, Plymouth.* In the laboratory they were kept in small groups in covered finger-bowls, with seaweed and a piece of cotton-wool soaked in sea water; the bowls were kept in semi-darkness. Like

* I am very grateful to the officers of the Plymouth Marine Laboratory for the special efforts they made to obtain animals extending over a wide range of body sizes.

Numanhoi (1933) I found that animals would survive under these conditions for a considerable time. To avoid complications due to possible sex differences only males were used in the experiments described; they reach a larger size than females.

Linear dimensions

Primarily the object of this research was to assess the relative values of 'body weight' and 'surface area' as standards for comparing the oxygen consumption of animals of different size. The 'surface area' it may be possible to measure, however, will differ considerably from the true 'surface area' (Ellenby, 1945*a*); what is measured is something proportional to the true surface. To use the two-thirds power of the body weight for estimating surface area would be to assume that the animals were constant in shape and specific gravity over their size range. If their shape is constant, however, the task is much simpler, for 'surface area' will then be proportional to the square of a linear dimension.

Accordingly, the 'length' and 'breadth' of experimental animals were measured; 'length' excluding uropods and antennae, and 'breadth' being the greatest width of the fourth segment. The animals were measured live as they are flaccid when dead, a lantern slide of a piece of millimetre ruled paper constituting both ruler and method of holding the animal; it could be swivelled round until squared up and the dimensions then read off to the nearest $\frac{1}{2}$ mm.

Pleopods of other animals were measured. The ventral ramus of the right third pleopod was mounted in Euparal, and the greatest distance across its short axis measured with a calibrated eyepiece micrometer. As the pleopod is normally held at right angles to the axis of the body, the dimension measured corresponds to the effective length of the beating organ, although, in fact, it is the morphological breadth.

Oxygen consumption

Animals were starved overnight prior to measurement of oxygen consumption. Measurements were made with an apparatus of the Haldane constant-pressure type already described (Ellenby, 1938) in which 1 cm. of the scale was equivalent to 0.0146 c.c. of dry oxygen at S.T.P. The pots, however, were considerably larger than in the earlier model and accommodated the animals on their floor. A tube cemented to each stopper was flanged and turned back at its distal end to form a shallow trough; a strip of Whatman no. 40 filter-paper wound round each tube rested in some potassium hydroxide in the trough and presented a large absorptive surface close to the animal.

The animals normally remained still in the humid atmosphere of the respirometer. In nature they tend to nestle quietly in crevices, and to encourage this during the respiration measurements a small cellophane shelter was provided in each pot. Each was rather like a table with two legs removed on one side; large holes were bored in the 'table-top' so that gaseous exchanges could take place freely. Experiments were carried out at $25 \pm 0.2^\circ$ C., each usually lasting an hour varying somewhat with the size of an animal. Animals were weighed on an Oertling automatic balance, and their length and breadth then measured as described.

Pleopod beat

Two series of measurements were made. The first series, at 25° C., was made on animals used in the respiration experiments. Each was dropped into a small glass cell of sea water immersed in the water-bath, and, after a few minutes acclimatization, the time for 20 beats was measured with a stop-watch; this was repeated two or more times.

Other animals were used for the second series, all of them in the same open dish of sea water at a room temperature of 15° C. Each animal was confined to a small numbered perforated cage and the cages shuffled after each determination. By this second method three determinations were made on each of twenty-five animals in less than an hour.

RESULTS

Body form and increasing body size

An animal may remain more or less of the same shape, and yet one part of it may change in proportions. For example, in *Carcinus moenas* (Huxley, 1932) the relationship of carapace length and carapace width remains constant over a wide range of

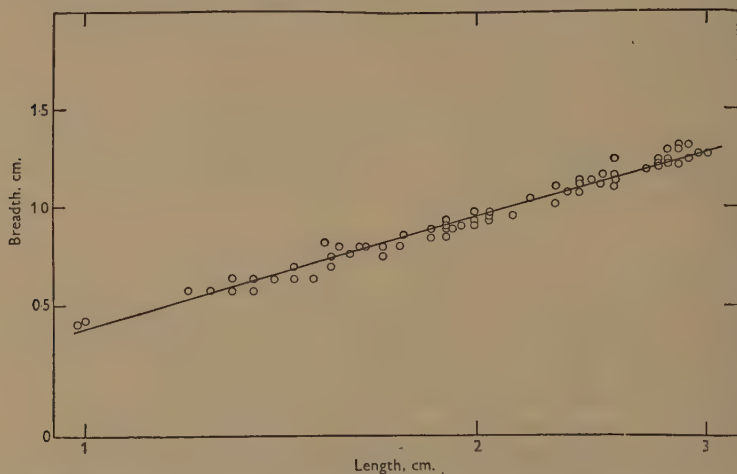


Fig. 1. Body length and breadth on a double logarithmic grid. The slope of the line does not differ significantly from 1.0, showing that both dimensions increase at the same rate.

body sizes; yet both frontal width and dentary width change in proportion to carapace length (Williams & Needham, 1941). In the present case the relationship of body length and width is considered, and then the relationship of body length and pleopod width; this will not show that there is no change in shape, but it will at least show whether it remains reasonably constant with increasing size.

Fig. 1, a double logarithmic plot, shows the relationship of length and breadth for eighty male *Ligia* ranging in body length from about 1 cm. to just over 3 cm. The

regression coefficient for the straight line fitting the data, calculated by the method of least squares, is $+1.027$; if the dimensions kept exactly in step the slope would be 1.0 . With a standard error of regression of ± 0.072 , P , the probability is, 0.7 , a very high value indeed; the difference from 1.0 is therefore not statistically significant.

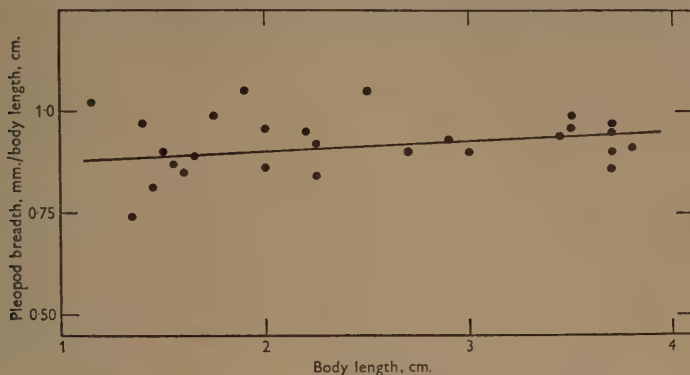


Fig. 2. Pleopod dimensions in relation to body size. Values of the ratio of pleopod breadth to body length show no significant tendency to change with increasing body size.

In Fig. 2 values for the ratio of pleopod breadth to body length are plotted against body length for twenty-eight animals. Change in shape would be shown by change in the values of the ratio, and the line which fits the data would not be horizontal; in fact, however, values keep fairly parallel to the abscissa, and analysis gives a regression coefficient of $+0.0147$. With a standard error of regression of ± 0.092 , P is greater than 0.1 , showing that the line does not differ significantly from the horizontal. In this respect also therefore, male *Ligia* show no significant change in shape with increasing size.

It may be of interest to mention another example of the constancy of form of this animal. For another purpose, the surface area of the pleopod was investigated. They were projected so that their images could be outlined. It was found that if the degree of magnification of the projector was adjusted so that in a series of animals it was in proportion to body length, the images of their pleopods coincided exactly.

Both analyses, then, show that it is reasonable to assume that male *Ligia* remain the same shape with increase in body size; surface area will therefore be proportional to the square of a linear dimension.

Oxygen consumption per unit of weight

Fig. 3 shows the values for oxygen consumption of eighty male *Ligia*, in mm^3 per mg. wet weight per hour, plotted against body weight; the animals range in body weight from 0.042 to 1.027 g. There is considerable variation in the data, but, clearly, on a weight basis, small animals consume very much more oxygen than large animals, the smallest animal examined having a rate of oxygen consumption about

three times that of the largest. The curve of Fig. 3 was fitted to values estimated from a linear regression equation calculated from the data by means of a logarithmic transformation. The analysis gave a regression coefficient of -0.274 , showing that oxygen consumption, per mg., is proportional to this power of the body weight, or that total oxygen consumption is proportional to the 0.726 power.

Body weight as such, therefore, is unsatisfactory as a standard; oxygen consumption is proportional not to weight itself but to its 0.726 power.

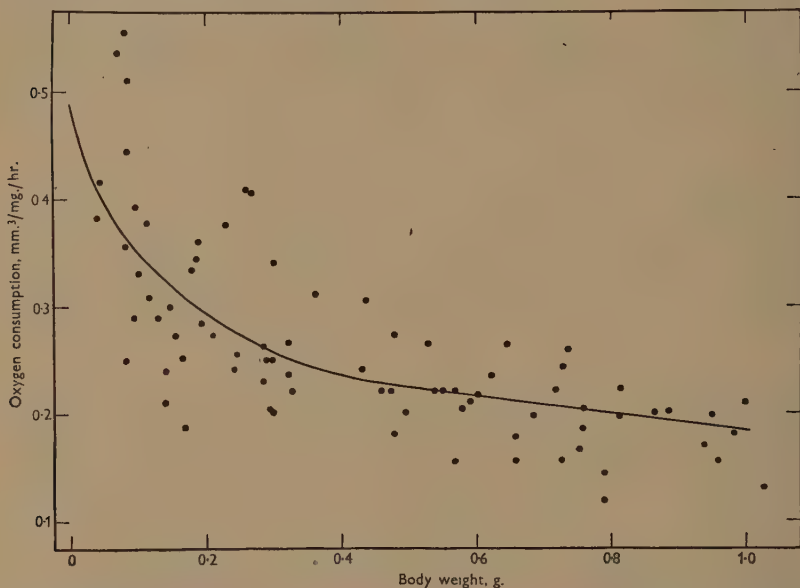


Fig. 3. Oxygen consumption and body weight, arithmetic plot; curve fitted by means of a logarithmic transformation which showed that oxygen consumption per mg. was proportional to the -0.274 power of the body weight.

Oxygen consumption per unit of surface

It has already been shown that surface area is proportional to length^2 ; accordingly values for oxygen consumption, per unit of length^2 per hour, were calculated, and these are plotted against body weight in Fig. 4. As in the case of the weight data, there is considerable variation in the data; but the values apparently keep parallel to the abscissa, that is, they show no tendency to increase or decrease with increasing body size. This the regression analysis confirms, for the regression coefficient is only 0.002 , or almost zero. In fact, the standard error of regression is ± 0.0019 , giving a value for P of 0.3 ; the line fitting the data therefore does not differ significantly from the horizontal.

Oxygen consumption, then, is proportional to 'surface area'. On the other hand, results evaluated on a weight basis show that it is proportional to the 0.726 power of

the body weight. In view of the variation in the data, the discrepancy between the two findings, for an animal of apparently constant shape, need not be taken too seriously; nor is it necessarily unresolvable. Surface area often varies as some power of body weight other than two-thirds; sometimes the value may be below two-thirds, as in the case of the bean aphid (Simanton, 1933) or fruit-fly prepupae (Ellenby, 1945*b*), but more frequently it is found to be above 0.7.

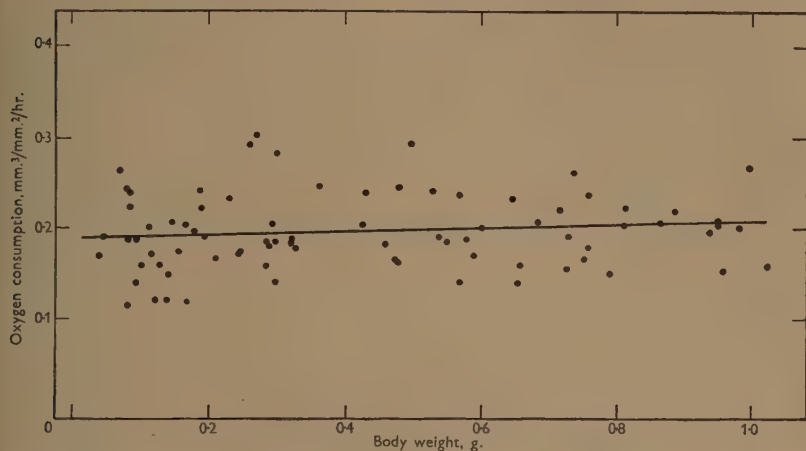


Fig. 4. Oxygen consumption and surface area. Values for oxygen consumption, per unit of length², show no significant tendency to change with increasing body size.

Surface area is proportional to length² if shape is constant and to body weight^{2/3} if both shape and specific gravity are constant. As shape is constant, 'volume' will be proportional to length³; substituting 'body-weight' for 'volume' will therefore show whether specific gravity is constant. Values for the ratio of body-weight to length³ are plotted against body weight in Fig. 5; they show no trend. As the regression coefficient is only 0.00003, test of its significance is hardly necessary. Clearly there are no grounds for assuming that specific gravity changes with the size of the animal.

As both shape and specific gravity are apparently constant, surface area will be proportional to the two-thirds power of the body weight. There is, therefore, an apparent contradiction in the fact that oxygen consumption has been shown to be proportional both to length², or 'surface', and also to the 0.726 power of the body weight. Fortunately, it is possible to estimate whether the difference is statistically significant.

The regression analysis carried out with the logs of values for oxygen consumption per mg. and body weight gave a regression coefficient of -0.274. Does this differ significantly from the value 0.333 necessary if the two-thirds power relationship is valid? Since the standard error of regression is ± 0.041 , t will equal 0.059/0.041, or 1.42; with 78 degrees of freedom, the value for P , the probability, lies between 0.1

and 0.2. There is therefore no statistically significant difference between the value obtained and -0.333 .

Pleopod beat

In Figs. 6 and 7 the time for a complete pleopod beat is plotted against body length, for experiments at 15 and 25° C. respectively. Comparison of the mean values for each curve gives a temperature coefficient of 1.36, a value somewhat lower than that

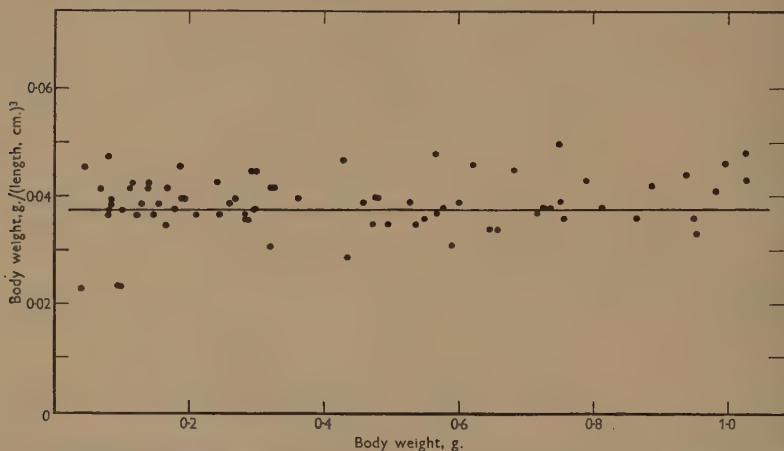


Fig. 5. Values for the ratio of body weight to the cube of body length show no significant tendency to change with increasing size showing that specific gravity is constant.

obtained for *L. exotica* (Numanhoi, 1933). The most interesting aspect of the results, however, is the variation in rate of beat with size of animal, pleopods of small animals beating faster than those of larger ones. Though the total oxygen consumption of a small animal is less than that of a large one, it is higher per unit of weight. In the case of the pleopod beat, however, the difference between animals differing in size is an absolute one. Oxygen consumption and pleopod beat vary at different rates with increasing body size.

D'Arcy Thompson (1942) has compared the rate of movement of a limb to that of a pendulum, the time of swing of which varies as the square root of the pendulum length; this comparison was first made, according to him, by the brothers Weber in 1836. The movement of a pleopod will, of course, be heavily damped by the water in which it is swinging. Nevertheless, it seemed interesting to consider pleopod beat from this point of view. Accordingly, values for time of swing and body length, the latter already shown to be proportional to 'pendulum length' (Fig. 2), were converted to logs and the regression coefficient calculated by the method of least squares. At 25° C., time of swing is proportional to $\text{length}^{0.59}$, and, at 15° C. to $\text{length}^{0.66}$. The first value does not differ significantly from 0.5, the value for a pendulum; the second value differs rather more and, although statistical analysis shows that the difference

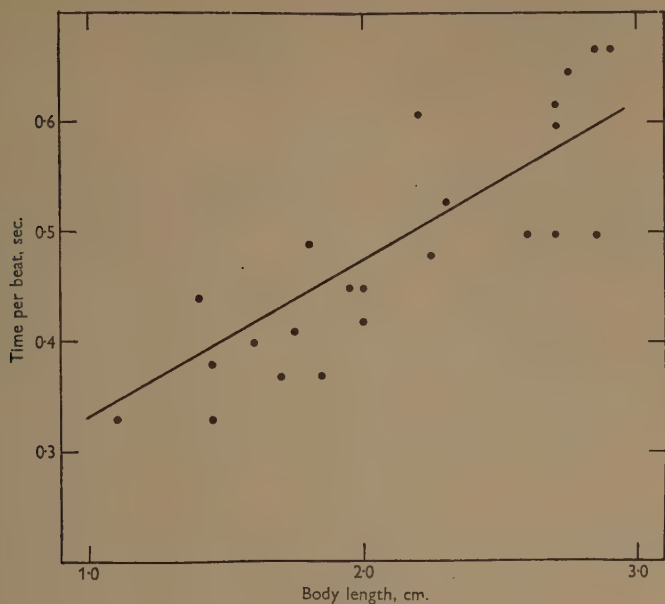


Fig. 6. Pleopod beat and body size, 15° C.

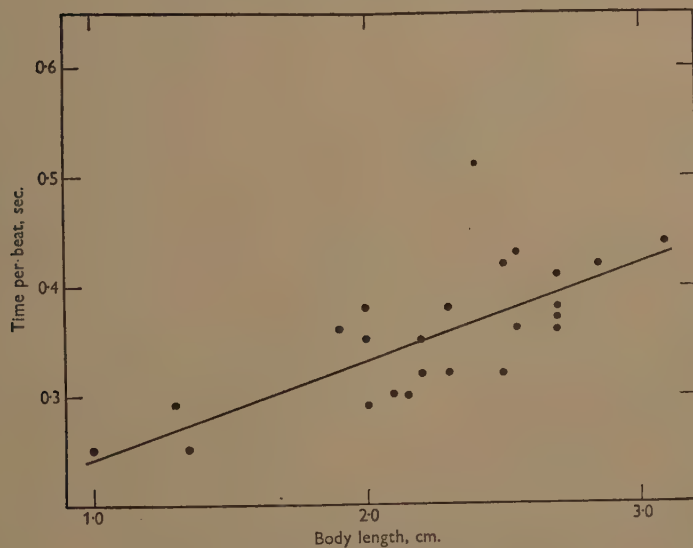


Fig. 7. Pleopod beat and body size, 25° C.

is not significant, it approaches very close to it, for P is only slightly greater than 0.05. Nevertheless, in spite of the damping, the *Ligia* data give surprising support to D'Arcy Thompson's contention that the pendulum theory, 'with proper and large qualifications', is 'substantially true'.

DISCUSSION

The results clearly show that the rates of both oxygen consumption and pleopod beat vary with the size of the animal. The total oxygen consumption of small animals is less than that of large ones, but they consume more per unit of weight; on the other hand, the pleopods of small animals beat actually faster than those of large animals. Pleopod beat varies very much like that of a pendulum, the time for a complete swing of which is proportional to the square root of pendulum length; the values actually obtained were 0.66 and 0.59 at 15 and 25° C. respectively, neither of which differed significantly from the 0.5 power. Total oxygen consumption is proportional to length² or to the 0.726 power of the body weight, but the latter value does not differ significantly from the two-thirds power.

There are examples from most invertebrate phyla where metabolism has been shown to vary with the size of the animal; in some cases it has been suggested that it varies with surface area. (For references see Wingfield, 1939; Whitney, 1942.) Weymouth *et al.* (1944) have recently investigated the rate of oxygen consumption of the kelp crab, *Pugettia producta*. Total oxygen consumption varied as the 0.798 power of body weight, 'weight-specific' oxygen consumption, that is, oxygen consumption per unit of body weight, as the -0.198 power of body weight. Considering the results of other workers on Crustacea, principally Montuori (1913), they show that, interspecifically, the results for twenty-three species show total oxygen consumption proportional to the 0.826 power, a value which does not differ significantly from 0.798 obtained for the kelp crab. The individual results used in the interspecific comparison must be taken with reserve, for they are derived from experiments carried out under different conditions, particularly of temperature; it is doubtful, for example, whether a temperature coefficient, calculated for temperatures up to 17° C., is valid for temperatures up to 24.5° C. But, nevertheless, the agreement between the exponents of body weight is remarkable. Apparently the relationship holds good both interspecifically, for mature individuals of different species, and intraspecifically, for individuals differing in maturity.

Kleiber (1947) considers the application of a power law in intraspecific comparisons of mature mammals differing in size. For mice, rabbits and dogs, the only forms for which material was available covering a sufficient size range, he concludes that it is reasonable to adopt the same standard for both intraspecific and interspecific comparison; but the same standard does not apply among mammals, for example, human beings, differing in maturity (see DuBois, 1936).

Though the values for the kelp crab and the other Crustacea do not differ significantly from each other, both differ significantly from a two-thirds power relationship.

The exact value of the exponent has some importance in relation to theoretical

considerations of factors influencing metabolic rate. The two-thirds power of the so-called surface-area law associated with the name of Rubner (1883) has long been applied in studies of the metabolism of mammals. There is, of course, no evidence that the actual surface of the animal is causally related to metabolic rate; indeed, the problem of the rabbit's ears, amusingly posed by Kleiber (1932), is almost sufficient on its own to dispose of such a view. Moreover, as shown by Kleiber (1932, 1947), Benedict (1938), Brody (1945) and many others, the metabolic rate of mammals is more nearly proportional to some power between 0.73 and 0.76, and Weymouth considers some such power to apply in the case of invertebrates. Although I do not believe that there is a causal surface relationship, some of the arguments used in dismissing a surface law seem faulty.

'Surface' is dismissed because the values obtained differ from two-thirds. I have already pointed out (Ellenby, 1945*b*) that the surface area of a series of animals need not be proportional to this power. The surface area (S) of any particular body will be proportional to the two-thirds power of its volume, or $S = KV^{\frac{2}{3}}$; as for a particular body, volume will be proportional to weight, $S = KW^{\frac{2}{3}}$. If the argument is extended from one particular body to a series of particular bodies of different sizes, there is no *a priori* reason why the same relationship should hold good; but it will if the shapes of the bodies are similar and if specific gravity remains constant. As Weymouth *et al.* (1944) point out, the two-thirds power is a mathematical fiction; in fact, in the comparatively few cases in which surface area measurements have actually been made values different from two-thirds have usually been obtained; for example, 0.63 and 0.60 for the German cockroach and the bean aphid (Simanton, 1933), 0.515 for *Drosophila* prepupae (Ellenby, 1945*b*), and values over 0.7 are common for mammals (Cowgill & Drabkin, 1927).

The rejection of a two-thirds power is justified if an alternative value is more suitable; but the rejection of a surface law is not justified unless surface area has actually been measured, or there is strong evidence that neither specific gravity nor body shape changes with body size. Such evidence is rarely advanced. In fact, it is well known that shape rarely remains constant and, as Meeh already showed for man in 1879, specific gravity may also vary with body size.

For *Ligia*, body shape and specific gravity do not change significantly with increasing size; surface area is therefore proportional to the square of a linear dimension. If rate of oxygen consumption is calculated per unit of length², it is found that the values are constant over the entire size range. Oxygen consumption is therefore proportional to 'surface area' estimated in this way. On the other hand, oxygen consumption was proportional to the 0.726 power of the body weight, a value much closer to the 0.74 derived by Weymouth from Benedict's data (1938), than the 0.798 of Weymouth *et al.* (1944), the 0.734 of Brody *et al.* (1934), or the three-quarters power of Kleiber (1947); but it does not differ significantly from two-thirds. Kleiber (1947) shows that it is impossible, with his mammal data, to distinguish statistically between a two-thirds power and 0.75 with a weight range less than ninefold. The *Ligia* body weights range from 0.04 to 1.03 g., a 25-fold increase; nevertheless, the two values do not differ significantly. This may be due to the large

variation in the data; but there is certainly no reason, at the moment, for assuming anything other than a surface relationship for *Ligia*.

It is interesting to note that values calculated per unit of length² agree among themselves somewhat better than values calculated on the basis of weight to the 0.726 power. The standard deviation, or the standard error of estimate, is about 24 % of the mean value in the first case, and about 33 % in the second case. This, of course, does not mean that one basis is more 'correct' than the other, for 'length' and 'weight' are measured by different techniques; but it is rather salutary, to say the least, to find that a technique in which length, measured with a millimetre rule with an error of about $\pm 2\%$, should provide a better basis for comparing rate of oxygen consumption than live weight determined with an accuracy of about 0.04 % with an expensive analytical balance.

Whatever the explanation of the decrease in rate of oxygen consumption per unit weight with increase in body size, there is no doubt that body size is one of the most important variables in metabolic studies. Weymouth and his colleagues estimate from their data that no less than 96 % of the variance in oxygen consumption is associated with variance in body weight and only 4 % with the variance in all other factors; they obtain very similar figures for the other Crustacea and mammals they consider. There is little doubt of the 'hopelessness of analysing other factors unless size is eliminated'.

In a series of papers beginning in 1936, Fox and his co-workers have considered 'activity' and 'metabolism' in relation to animal distribution. A large number of poikilothermal animals from cold waters are compared with related forms from warmer water, occasionally with members of the same species. Rates of oxygen consumption are measured and also the rates of various other activities used as indicators of 'general activity'. It is claimed (Fox & Wingfield, 1937) that when two species within a genus are compared, the relation of oxygen consumption to temperature can be expressed by a single curve. At the higher temperatures at which it lives, an English species consumes more oxygen than an arctic species of the same genus in the colder water of its habitat, although the locomotory activity of the former is no greater. 'It is suggested' (Fox, 1936) 'that the similar locomotory activities of the two species require approximately equal amounts of oxygen, but that the non-locomotory oxygen consumption of the warm-water species is higher than that of the cold-water species. . . . The respiratory movements of the Crustacea formed a striking contrast to their oxygen consumption. The relation of rate of respiratory movements to temperature, for each of three pairs of comparable species did not form a single curve but two roughly parallel curves; at the temperature of its habitat, the respiratory appendage of each English species moved no faster than that of its arctic cousins living in colder water. This was true even within a single species of prawn (*Pandalus montagui*) inhabiting the two regions.'

The distinction between the 'activities' measured and oxygen consumption can hardly be sustained; activities such as heart beat, or movements of a respiratory appendage, are surely 'basal' rather than indicators of 'general activity'. Moreover, rate of pleopod beat and oxygen consumption have been shown, for *Ligia*, to be

activities taking place on different planes. The most serious question, however, is the validity of the method used to compare the rate of oxygen consumption or heart beat of two different organisms. This is hardly considered. Oxygen consumption is calculated per unit of weight, and the various activities are recorded on a time basis; these standards are perfectly suitable, but only if other things are equal. Rate of movement of the similar respiratory appendages of a pair of animals will be reasonable indicators of the amount of work involved in moving the appendages; but if the appendages are of different sizes, the comparison is much more difficult and some basis for the comparison must be found.

It is well known that northern forms are usually larger than similar forms from warmer waters (see Sverdrup, Johnson & Fleming, 1942); but Fox rejects the possibility that the metabolic differences may be due to the size difference. In a footnote (1936, p. 950) he states: 'It might be suggested that the smaller size of the English species noted above was responsible for their greater oxygen consumption, but within any one species I found no correlation between individual size and oxygen intake...' Correlation coefficients are cited ranging from -0.2 to $+0.13$, and a similar value is also given in later experiments with other forms (Fox & Wingfield, 1937). But the correlations evaluated appear to be between oxygen consumption, per unit of weight, and body weight; these would be spurious. As pointed out by Weymouth *et al.* (1944), body weight will already have entered into the ratio with which it is correlated; if there is a positive correlation between total oxygen consumption and body weight, as seems likely, the resulting negative correlation for the 'weight-specific' rate will be lowered. But, in any case, as the numbers are small, comparison of correlation coefficients alone is hardly adequate for such important conclusions. Moreover, it is doubtful, to say the least, whether, even if the correlation test is accepted, it is sound to extrapolate from one group to another differing from it in size much more than the range of size within the original group.

It is interesting to compare the values obtained in a similar analysis carried out with the *Ligia* data. In order to compare them with those of Fox, the correlation coefficient was calculated for the first fifteen values for oxygen consumption per milligram, a number similar to the number in Fox's tests. This gave a coefficient of $+0.10$. On the other hand, when the correlation coefficient was calculated for all the data, the value obtained was -0.69 . Clearly, a size effect in a small group of animals which do not differ greatly in size would have great difficulty in manifesting itself. If it is incorrect to extrapolate from a restricted size range to the whole size range, it is even more incorrect to extrapolate from a restricted size range of one form to another form of larger size.

Fox claims that the 'activity' of related forms from northern and southern waters is apparently the same, each at its own normal temperature, and that this is shown by the rate of movement of their respiratory appendages, etc. For a pair of forms, therefore, two parallel curves relate rate of beat and temperature, while the results for both forms will fit a single curve relating temperature and rate of oxygen consumption per gram. But the northern forms are larger. If oxygen consumption

varies with body size and is proportional to surface or some such function of body weight, oxygen consumption per unit of surface will be higher for the northern forms than for corresponding southern forms, as, per gram, it is claimed that they have the same consumption. A pair of forms will therefore give two curves relating oxygen consumption per unit of surface and temperature, just as in the case of the relationship for rate of movement of a respiratory appendage. There will therefore be no grounds for assuming that the warmer water species show environmental adaptation in having a higher rate of 'non-locomotory' metabolism.

That a size factor may, in fact, be operating to obscure other relationships is suggested by a comparison of the different English species of Crustacea (Fox, 1936; Fox & Wingfield, 1937). Four species were examined at 10° C., *Pandalus montagui*, *Pontophilus norvegicus*, *Pandalina brevirostris* and *Spirontocaris cranchi*. With mean body weights of 2.2, 0.3, 0.15 and 0.04 g. respectively, their rates of oxygen consumption, in mm.³/g./hr., are 106, 87, 119 and 141; values therefore tend to increase with decreasing body weight. But there is no such tendency in the case of three corresponding northern forms examined at 6.5° C.; with body weights of 9.2, 4.4 and 1.8 g. the rates of oxygen consumption are 75, 82 and 77. *Pandalus montagui*, which occurs in both localities, has a mean weight at Kristineberg of 3.2 g. and at Plymouth of 2.2 g.; the rates of oxygen consumption at 10° C. are 123 and 106 respectively. This form, however, does not fit Fox's general scheme; it gives 'parallel' oxygen consumption curves with temperature, and he suggests that it has a higher locomotory metabolism at Kristineberg than at Plymouth.

Fox claims that the rate of scaphognathite beat does not vary with the size of the animal. As before, however, the claim is based on a correlation test on a small group of limited size range. This is supported for *Pandalus borealis* by Abercrombie & Johnson (1941), but it is interesting to note that their specimens varied in size only from 11 to 16 cm.; clearly, if variation between individuals is large, as they in fact found, a size effect could easily be obscured. It must again be pointed out that the apparent absence of a size effect in a group of animals of restricted size is no valid basis for the assumption that there is no such effect between two species differing in size to a much greater extent.

As recently shown for fish by Peiss & Field (1950) there may be striking metabolic adaptations to life in cold waters; but Fox's comparisons are unfortunately invalidated by lack of consideration of the factor of body size. This is also true of the interesting investigation of the response of ephemerid nymphs from different habitats to change in oxygen tension (Fox, Wingfield & Simmonds, 1937a). It is most unfortunate that more attention was not paid to control of the factor of body size, particularly in view of the care taken in previous papers in this series (Fox & Simmonds, 1933; Fox, Simmonds & Washbourn, 1935). Their general conclusion that forms from fast-flowing streams respond to a reduction in oxygen consumption much more rapidly than forms from less highly oxygenated habitats may prove to be justified, but it cannot be said that this paper has established the point.

Rate of oxygen consumption was measured for each form at a number of different oxygen tensions; but different specimens were used at each oxygen tension, and,

unfortunately, the mean weight often varied from tension to tension, sometimes in a systematic fashion.

Baetis sp. appears to show the most rapid response to lowered oxygen tension. At oxygen concentrations of 14.6, 12.3, 7.7, 5.3 and 4.0 c.c./l., the rates of oxygen consumption, in mm.³/g./hr., were 4000, 4100, 2700, 1670 and 1350 respectively. Mean dry weights for the specimens examined at these tensions were, respectively, 10.0, 11.0, 15.7, 21.7 and 17 mg.; weight, therefore, increases more or less regularly with decreased tension and oxygen consumption. The position is slightly different in the case of *Leptophlebia vespertina*, which appears to show a response only at the two lowest levels of oxygen tension. Mean weights, in order of decreasing oxygen tension, were 27.7, 33, 37, 27, 41.2 and 54 mg.; size differences are therefore slight, until the two lowest levels of oxygen tension, where the rate of oxygen consumption also shows the most striking fall.

The size distribution is satisfactory in the case of *Ephemera vulgata*, a form which shows marked response to falling oxygen tension; but this species lives buried in mud and the response is not considered adaptive.

Clearly it is impossible to obtain a clear picture of the true response to lowered oxygen tension, for the differences of the mean weight of the animals in the different groups would itself influence the level of oxygen consumption.

A better conclusion to this section could hardly be found than that of Dreyer, Ray & Ainley-Walker (1913): 'In recent years it has become increasingly evident that many of the most important problems of physiology and experimental pathology cannot be investigated in a satisfactory manner until accurate data have been made available regarding the quantitative differences which are exhibited by the organs, tissues and fluids of normal animals of different species and varying weights. Results obtained with animals of different species and any given weight cannot be applied even within one and the same species, to yield conclusions regarding animals of different weight until it has been determined with precision how the various organs and tissues of the body are related to the size of the individual. Moreover, it will only be possible to compare one species with another, or to apply the results deduced from any given species to any other species of animal, until we have established some kind of quantitative correlation between the measurements in the different species.'

SUMMARY

1. Male *Ligia oceanica* were used in an investigation of the relationship of body size to rate of oxygen consumption and pleopod beat.
2. Animals varied in weight from 0.04 to 1.03 g. and from 0.95 to 3.1 cm. in length.
3. Body shape does not change significantly over the size range, for length and breadth both increase at the same rate, and pleopod dimensions bear a constant relation to body length.
4. Specific gravity also is constant, for the relation of body weight to the cube of body length shows no trend with increasing size.

5. Oxygen consumption per gram decreases with increasing size and is proportional to the -0.274 power of body weight. Total oxygen consumption is therefore proportional to the 0.726 power of body weight; but this value does not differ significantly from two-thirds.

6. As shape is constant, surface area is proportional to the square of a linear dimension. It is shown that oxygen consumption per unit of length² is constant over the size range. Although body length was measured far less accurately than body weight it is shown that it assesses 'body size' more accurately.

7. Rate of pleopod beat was measured at 15 and 25° C.; it decreases with the size of the animal. At 15° C. time per beat varies as the 0.66 power of body length, and at 25° C. as the 0.59 power; neither of these values differs significantly from 0.5 . Despite the fact that pleopod movement is heavily damped, the rate therefore varies like that of a pendulum.

9. The work of Fox (1936-9) and Fox *et al.* (1937*a*) on the rate of oxygen consumption of animals from cold and warmer seas and from different habitats is considered. It is suggested that many of their comparisons are invalidated by differences in body size of the animals concerned, and that, in relation to environment, no basis, theoretical or experimental, has been established for a distinction between 'non-locomotory' and 'activity' metabolism.

REFERENCES

- ABERCROMBIE, M. & JOHNSON, M. L. (1941). The effect of temperature on the respiratory movements and viability of a cold-water prawn, *Pandalus borealis*. *Proc. Zool. Soc. Lond. A*, **111**, 87-99.
- BENEDICT, F. G. (1938). Vital energetics. A study in comparative basal metabolism. *Publ. Carneg. Instn*, no. 503.
- BRODY, S. (1945). *Biogenetics and Growth*. Reinhold.
- BRODY, S., PROCTOR, R. C. & ASHWORTH, U. S. (1934). Growth and development with special reference to domestic animals. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight. *Res. Bull. Mo. Agric. Exp. Sta.* no. 220, pp. 3-40.
- COWGILL, G. R. & DRABKIN, D. L. (1927). Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. *J. Amer. Physiol.* **81**, 36-61.
- DREYER, G., RAY, W. & AINLEY-WALKER, E. W. (1913). The size of the aorta in warm-blooded animals and its relationship to the body-weight and the surface area as expressed as a formula. *Proc. Roy. Soc. B*, **86**, 39-55.
- DUBOIS, E. F. (1936). *Basal Metabolism in Health and Disease*. Baillière, Tindall and Cox.
- ELLENBY, C. (1937). Relation between body size and metabolism. *Nature, Lond.*, **140**, 853.
- ELLENBY, C. (1938). Metabolic rate of early vestigial and wild-type prepupae of *Drosophila melanogaster* in relation to genotype, sex and size. *Proc. Zool. Soc. Lond. A*, **108**, 525-38.
- ELLENBY, C. (1945*a*). Oxygen consumption of prepupae of *Drosophila melanogaster* Meigen, in relation to the surface area of the puparium. *J. Exp. Biol.* **21**, 39-45.
- ELLENBY, C. (1945*b*). Empirical formulae and the surface area of puparia of *Drosophila melanogaster* Meigen. *J. Exp. Zool.* **98**, 23-33.
- FOX, H. MUNRO (1936). The activity and metabolism of poikilothermal animals in different latitudes. I. *Proc. Zool. Soc. Lond. A*, **106**, 945-55.
- FOX, H. MUNRO (1939). The activity and metabolism of poikilothermal animals in different latitudes. V. *Proc. Zool. Soc. Lond. A*, **109**, 141-57.
- FOX, H. MUNRO & JOHNSON, M. L. (1934). The control of respiratory movements in crustacea by oxygen and carbon dioxide. *J. Exp. Biol.* **11**, 1-10.
- FOX, H. MUNRO & SIMMONDS, B. G. (1933). Metabolic rates of aquatic arthropods from different habitats. *J. Exp. Biol.* **10**, 67-74.

- FOX, H. MUNRO, SIMMONDS, B. G. & WASHBOURN, R. (1935). Metabolic rates of ephemerid nymphs from swiftly flowing and from still waters. *J. Exp. Biol.* **12**, 179-84.
- FOX, H. MUNRO & WINGFIELD, C. A. (1937). The activity and metabolism of poikilothermal animals in different latitudes. II. *Proc. Zool. Soc. Lond. A*, **107**, 275-82.
- FOX, H. MUNRO, WINGFIELD, C. A. & SIMMONDS, B. G. (1937a). The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. *J. Exp. Biol.* **14**, 210-18.
- HUXLEY, J. S. (1932). *Problems of Relative Growth*. Methuen.
- JOHNSON, M. L. (1936). The control of respiratory movements in Crustacea by oxygen and carbon dioxide. II. *J. Exp. Biol.* **13**, 467-75.
- KLEIBER, M. (1932). Body size and metabolism. *Hilgardia*, **6**, 315-53.
- KLEIBER, M. (1947). Body size and metabolic rate. *Physiol. Rev.* **27**, 511-41.
- MEEH, K. VON (1879). Oberflächenmessungen des Menschlichen Körpers. *Z. Biol.* **15**, 425-58.
- MONTUORI, A. (1913). Les processus oxydatifs chez les animaux marins en rapport avec la loi de superficie. *Arch. Ital. Biol.* **59**, 213-34.
- NICHOLLS, A. G. (1931). Studies on *Ligia oceanica* L. I. A. Habitat and effect of change of environment on respiration. B. Observations on moulting and breeding. *J. Mar. Biol. Ass. U.K.* **17**, 655-74.
- NUMANHOI, H. (1933). Effects of temperature on the frequency of the rhythmical beatings of the pleopods of *Ligia exotica*. *J. Fac. Sci. Tokyo Univ.* **3**, 217-25.
- NUMANHOI, H. (1937). Effect of temperature upon the amplitude of the pleopodal beatings in *Ligia exotica*. *J. Fac. Sci. Tokyo Univ.* **4**, 377-82.
- PEISS, C. N. & FIELD, J. (1950). The respiratory metabolism of excised tissues of warm- and cold-adapted fish. *Biol. Bull. Woods Hole*, **99**, 213-24.
- RUBNER, M. (1883). Ueber den Einfluss der Körpergrösse auf Stoff- und Kraftwechsel. *Z. Biol.* **19**, 536-62.
- SIMANTON, W. A. (1933). Determination of the surface area of insects. *Ann. Ent. Soc. Amer.* **26**, 247-54.
- SVERDRUP, H. V., JOHNSON, M. W. & FLEMING, R. H. (1942). *The Oceans, Their Physics, Chemistry, and General Biology*. Prentice-Hall.
- THOMPSON, D'ARCY W. (1942). *On Growth and Form*. Cambridge.
- WEYMOUTH, F. W., CRISMON, J. M., HALL, V. E., BELDING, H. S. & FIELD, J. (1944). Total tissue respiration in relation to body weight. A comparison of the kelp crab with other crustaceans and with mammals. *Physiol. Zool.* **17**, 50-71.
- WHITNEY, R. J. (1942). The relation of animal size to oxygen consumption in some fresh water turbellarian worms. *J. Exp. Biol.* **19**, 168-75.
- WILLIAMS, G. & NEEDHAM, A. E. (1941). Metric variations in populations of *Carcinus moenas*. *J. Mar. Biol. Ass. U.K.* **25**, 261-81.
- WINGFIELD, C. A. (1939). The activity and metabolism of poikilothermal animals in different latitudes. IV. *Proc. Zool. Soc. Lond. A*, **109**, 103-8.

THE EXPERIMENTAL INVESTIGATION OF NAVIGATION IN HOMING PIGEONS

By G. V. T. MATTHEWS

Department of Zoology, University of Cambridge

(Received 26 March 1951)

(With Twelve Text-figures)

INTRODUCTION

There are two main problems in bird navigation, concerned respectively with natural migration journeys and with 'homing' to an area after being artificially transported away from that area. The latter more complete problem is the concern of the present work. The term 'navigation' is used here in the sense of the dictionary definition, 'the art or science of directing the movements of vessels on the sea . . . or in the air', and this is taken to exclude 'pilotage' which is defined as 'the steering of ships . . . wherever local knowledge is required'. Thus if it should be shown that birds only returned regularly from areas already known to them, and that returns from unknown areas were attributable to chance wanderings, then the term 'navigation' would have to be dropped. One school of thought to-day holds this opinion with some minor concessions. The other holds that at least some of the facts of homing cannot be dismissed on this basis, and a number of explanatory theories have been put forward. It is not the purpose of the present paper to provide full chapter and verse for the controversy that has been going on for more than eighty years. Griffin (1944) provides an adequate summary, and Matthews (1950) has discussed it in rather more detail.

One of the main causes of this prolonged debate has been the dearth of detailed, critically assembled facts. In particular, the difficulty of delimiting the area known to any particular bird has resulted in ambiguous interpretations of such facts as we do possess. This difficulty is much easier to overcome in the case of homing pigeons, particularly with young birds only a few months old. It is therefore surprising that this obvious experimental animal has been so little used by investigators. There is, of course, a vast popular literature, mostly highly uncritical. Accounts of the usual training and performances have been given, among others, by Riviere (1923), Knieriem (1943) and Nicol (1945). Field tests of a general nature have been carried out by Thauzies (1909), Riviere (1929), Gibault (1930, 1933), Grundlach (1932), Heinroth (1941) and Platt & Dare (1945), with conflicting results. A number of other experimenters have used pigeons to test particular theories, taking small numbers of unknown strains and histories, and employing them with little knowledge of their capabilities under normal conditions.

The present work was therefore undertaken first to provide a quantity of statistically satisfactory experimental data on the homing behaviour of pigeons under

various conditions. Secondly, it was intended to investigate some of the theories of special navigational ability, if a straightforward explanation of the accumulated facts was not possible.

MATERIAL AND METHODS

It was necessary to rear large numbers of pigeons from proven racing stock, to use them in their first year, operating from a well-equipped loft where they were carefully handled. Field Station facilities were not then available, and the very essential breeding, rearing and initial training was carried out by three breeders of long standing with large establishments of successful strains, viz. L. A. Meredith at Easton, Norwich, in 1948 (25 birds), P. Cope at Duxford, Cambridge (34 birds), and L. R. Duke at Sawston, Cambridge (26 birds) in 1949, and P. Cope at Duxford (73 birds) in 1950.

The birds were hatched in two 'rounds', early in March and in Mid-April, so that all were 3-4 months old when the experiments were begun. By then they were accustomed to handling, travelling in baskets, re-entering the loft on their return and the other incidentals of the work, by several releases in groups up to 10 miles from the loft in one 'training' direction. A series of controlled experimental releases were then carried out.

The birds were fed on the afternoon prior to the test. They were then taken in a closed van (except for tests, A and B, in which rail transport was used) to Cambridge, and then on to the release point in the early hours of the morning, arriving about 0500 G.M.T. The greater part of the journey was thus done in the dark, and in addition the baskets containing the birds were covered with opaque black cloth. Water was provided but no food. On release, therefore, these young birds were required to exhibit a very simple form of homing—to 'home plus food'. No sexual motives governed their return.

Release points were chosen to give a clear all round view, no trees or buildings near enough to block out a pigeon flying at 100 ft. up to two miles away. The latter was found to be the approximate limit for following a single bird in 16×40 binoculars.

Each bird was tossed straight up into the air, the liberator facing in different directions during the course of releases. The bird was then followed in the binoculars until it had vanished from sight, when the next was released. In this way, in most of the experiments, the birds were operated as individuals and complication of results by group effects was avoided. As there is a strong tendency for single birds to join up with any strong-flying group of pigeons, no releases were made on Saturdays, or other days on which mass pigeon-racing was in progress.

The straight-line homing speeds of birds returning on the day of release were calculated. These, together with the proportion of stragglers and losses, form a useful index of success on any one test. Winds and other meteorological conditions must be considered when comparing one test with another. Small differences in homing speed may be due to actual differences in flight speed, but in the main will reflect the extent of deviation from the straight track home, or of halts by the way.

As each bird was lost from view in the binoculars, its bearing was noted. This 'Vanishing Point' is not necessarily representative of the course being flown, since the majority of birds, having circled once or twice, make off in one general direction, at the same time flying a series of wide zigzags or horizontal loops. The vanishing point is generally reached when the bird turns end-on to the observer, and is therefore likely to indicate the limits of the sideways search about the general line of flight. As an individual index the vanishing point may thus be somewhat misleading, but with a number of birds, a departure 'fan' is obtained whose orientation may be interpreted with confidence. Throughout the series of tests this index bore a general positive relation to homing success. Thus for all birds released singly more than 48 miles from the loft we have:

Vanishing point	Sorties	Homing speed	Returned 2nd day (%)	Stragglers and lost (%)
Within 30° of home line	222	Av. 24.5	12	9
More than 30°	363	Av. 19.8	9	21

The time from release to vanishing would depend partly on atmospheric conditions (although visibility was reasonable in all tests), on the attitude of the bird relative to the observer and on any tendency to wait for a companion. It has, however, been called 'Orientation Time', and this would seem to be justified as there is a positive correlation with the accuracy of the initial direction taken:

Orientation time (min.)	Sorties	Vanishing points within 30° of home line (%)
1½-3½	244	45
-5½	168	36
Over 5½	173	30

Similarly, a positive correlation with homing success can be demonstrated.

For the great majority of sorties these three indices of performance are available, showing the degree of uncertainty on release, the accuracy of the initial course and the success in regaining home. They are represented in histogram form for the important tests in Figs. 2-12, the detailed information being too bulky to include. That for 1948 and 1949 is available elsewhere (Matthews, 1950).

For ease of subsequent reference a list of the tests carried out is given in Table 1, the results and interpretations being discussed later as appropriate. The relation of the release points to home is shown in Fig. 1. This paper is thus based on the performances of 158 individual pigeons, in a total of 902 sorties. Only 153 of the latter were in groups of three to eight birds.

BEHAVIOUR OF PIGEONS WHEN RELEASED ON A 'TRAINING LINE'

It has been known for centuries that fewer losses and faster returns will result when pigeons are 'trained' by being released at increasing distances in one general direction only. All pigeon racing is based on this procedure, likewise the use of pigeons as

Table 1

Test	Date	No. of birds	Distance and bearing of home
1948: A	4. viii	24	55 miles 052° T.
B	18. viii	9	55 miles 052° T.
1949: 1	17. vii	50 (in groups)	24 miles 161° T.
2	21. vii	60	24 miles 161° T.
3	24. vii	18 (in groups)	
		38	49 miles 159° T.
4	27. vii	53	49 miles 159° T.
5	5. viiii	52	78 miles 160° T.
6	10. viiii	48	78 miles 160° T.
7	17. viiii	45	127 miles 158° T.
8	29. viiii	47	79 miles 070° T.
9	4. ix	33	69 miles 345° T.
1950: 10	11. vii	66 (in groups)	25 miles 164° T.
11	18. vii	61	25 miles 164° T.
12	25. vii	10	49 miles 085° T.
13	25. vii	44	53 miles 157° T.
14	1. viiii	43	
		3 (in group)	53 miles 157° T.
15	4. viiii	10	62 miles 079° T.
16	4. viiii	32	87 miles 154° T.
17	10. viiii	33	87 miles 154° T.
18	18. viiii	35	130 miles 161° T.
18a	18. viiii	8 (in group)	49 miles 143° T.
19	22. viiii	16	
		8 (in group)	13 miles 186° T.
20	24. viiii	30	81 miles 070° T.
20a	24. viiii	10	53 miles 064° T.
21	1. ix	16	68 miles 344° T.

message carriers. As a first step it was therefore necessary to investigate the factors governing this technique, and particularly to find out whether a tendency to fly in the one particular direction was established, and if so how the birds recognized that direction. There was the added advantage that such a procedure resulted in most of the birds becoming used to flying long distances without covering more than a narrow tract of country.

A series of tests were therefore carried out on one line bearing north-north-west from the loft. In the first the birds were released in groups, thereafter as individuals. They were released a second time at each stage before proceeding to the next. The distance between stages was successively increased, for instance, from 15 to 28, 34 and 43 miles in 1950, and similarly in 1949.

A general orientation of the departure 'fan' about the home/training direction could be discerned, and became clearer with each new stage up the line, an increasing proportion of birds being with a narrow arc:

Birds within 30° of home line

1949	Test 3	27%	1950	Test 13	21%
	5	50%		16	50%
	7	54%		18	53%

Also see and compare Fig. 2 with Fig. 4, and Fig. 3 with Fig. 9. The average orientation time varied about 5 min. without showing a steady decrease, suggesting

that the orientation process was a fairly constant one. The average speed of return increased through the series, e.g. 17.8, 20.2 and 30.9 m.p.h. in tests 3, 5 and 7. This reflects an increase in flying speed and strength, in confidence and 'desire' to reach home, and in the proportion of the track previously flown over.

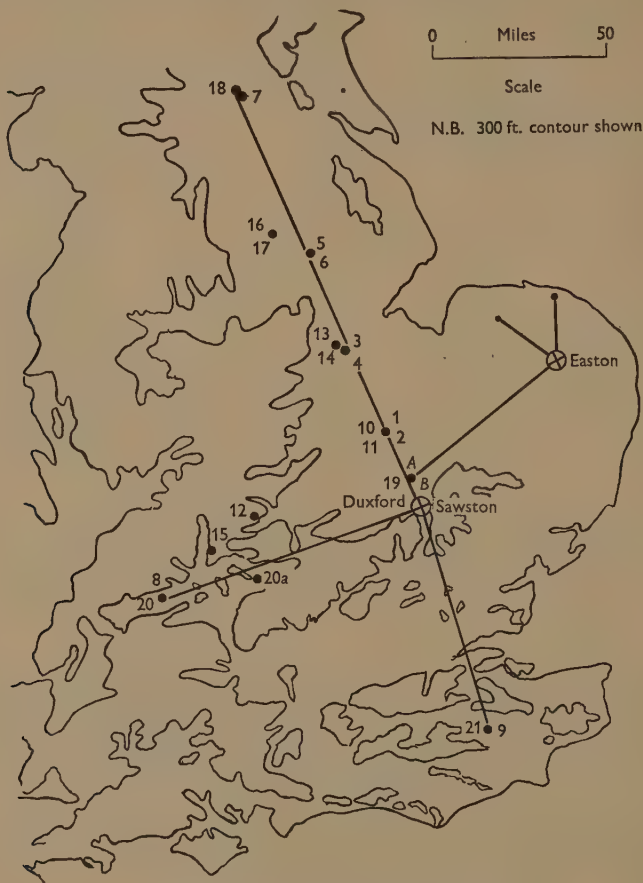


Fig. 1. Relation of release points to home lofts.

In the conditions under which pigeon races are carried out—mass releases of birds of mixed histories and training—leadership by experienced birds and other group effects undoubtedly play a part. As the present tests were expressly designed to eliminate these factors nothing much can be said on this score. In Test 3, however, eighteen birds were released in three groups, the remainder singly. The groups were lost from sight twice as quickly (despite their greater visibility) as the

single birds, and closer to the home direction (11° , 17° and 33° off, v. 27% within 30°). They also returned faster, 28.0 v. 17.8 m.p.h. The group effects resulting in this superior performance probably include the satisfaction of the desire for company, competitive striving, and cancelling out of diverging flight lines (groups rarely circle, and make off with few changes of direction). That some birds will follow the lead of their more confident fellows is suggested by the performance of these

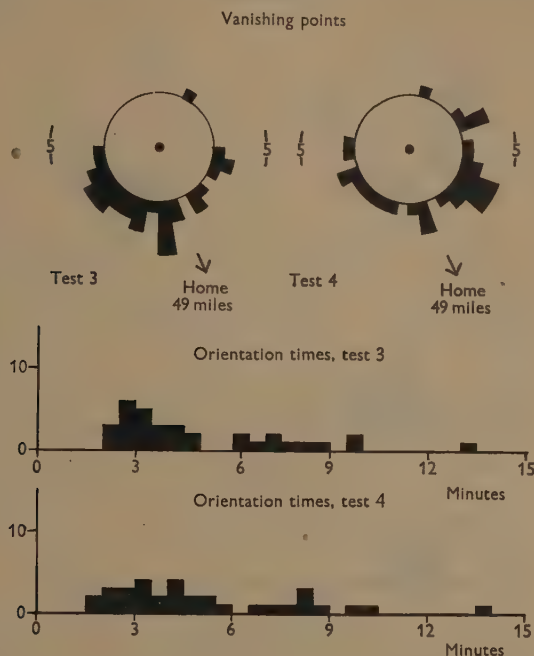


FIG. 2. Effect of overcast skies on a repeat release from a point on the training line.

grouped birds when released singly at the same point in Test 4. Ten reached vanishing point in 2.8–5.8 min., but seven others were very slow to depart, taking from 8.2 to 31.0 min. It seems probable that the latter had 'gone with the crowd' on their first release and had difficulty in picking up a line of flight on the second occasion.

The arrangement of these unidirectional releases made it most unlikely that any pigeon had been to a release point prior to the first test there. It also seems probable that the fairly moderate intervals between successive stages would be sufficient to prevent the viewing of known horizons. The pigeons flew relatively low, 100–200 ft., giving a horizon at 12–18 miles. In Test 5 owing to a strong head wind they flew very low, 30–70 ft., with a horizon at 7–10 miles. Yet a good concentration of vanishing points was still recorded in the home direction. Similarly, the coast line was

sufficiently distant, and its nearest points had no constant orientation relative to the training line.

The present results suggest that pigeons do not possess a 'photographic' memory of the general features of the landscape. When released a second time at the same point, the concentration of vanishing points may show some, though no very great,

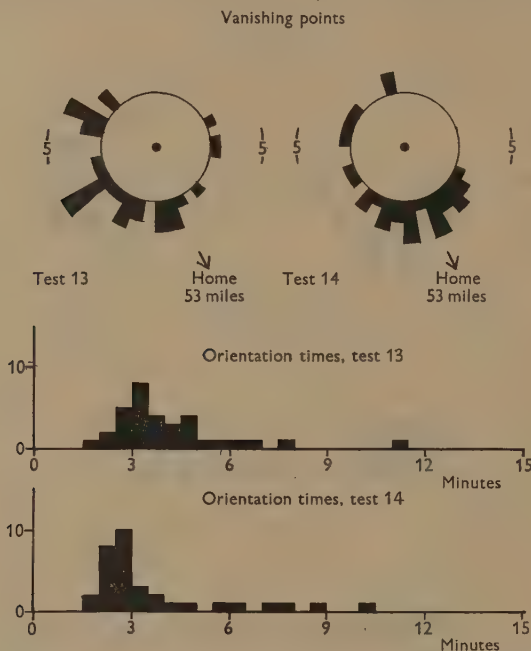


FIG. 3. Effect of overcast skies on an initial release from a point on the training line.

improvement, e.g. Tests 5 and 6, 45% v. 55% within 30°. In other cases there was no improvement, e.g. Tests 16 and 17, both had 57% within 30°, or a poorer performance may result, e.g. Tests 3 and 4, 33% v. 24%. It might be added here that pigeons have shown themselves to be slow learners in laboratory tests involving the use of visual clues (Matthews, 1950). It is probable, however, that general features of the landscape may influence the initial flight direction, especially in the early stages of training. Thus a low ridge lay to the south of the release point in Tests 10 and 11, while in Test 13 a similar ridge lay to the west. Fig. 3 suggests that a number of birds were attracted to the west in the latter test.

Overcast skies were found to have some effect on orientation in the training direction. In 1949, thirty-three birds were released singly in both Tests 3 and 4, and in 1950 another thirty-three were released singly in both Tests 13 and 14. All were thus in the same stages of training and were released in the same landscape, but with

widely differing cloud conditions. Test 3 was made in bright sunshine with 2/10th to 5/10th cloud, but in Test 4 the sky was overcast and the sun only visible very occasionally as a shrouded disk. Despite the presumed assistance of previously seen landmarks the birds gave a poorer performance in orientating on their second release. In 1950 the *first* release was made with the sky overcast and the sun infrequently visible as a disc, while the repeat release was in bright sunshine with 2/10th to 6/10th cloud. With this sequence a considerable improvement resulted. Results for the two sets of tests were:

Test	Orientation time	Within 30° of home
3	5'1	11
4	5'5	8
13	4'1	8
14	3'7	18

They are shown graphically in Figs. 2 and 3.

Schneider (1906) suggested that, as racing pigeons were usually released early in the morning, the position of the sun in azimuth would provide a relatively constant directional clue. Fixed-angle learning is within a pigeon's capacity, as it has been shown in laboratory experiments (Matthews, 1950) that they could learn to go to food placed at 090°, 180° and 360° to an artificial sun. Test 7 was designed to test this theory. The release points in Tests 1-6 and 7 (see Fig. 1, Table 1) were all within a degree or two of a straight line from the loft. Further, each individual pigeon was released as nearly as possible at the same Greenwich mean time in each of Tests 1-6. The *maximum* deviation from the individual mean time of release varied from 2 to 33 min., with an average value of 12 min. In all tests (except Test 4, a repeat) the weather was sunny with well-broken cloud. In Test 7, twenty-two birds were released 6 hr. earlier or later than their previous mean time of release, and twenty-three control birds were released at their normal time. In the course of 6 hr. the sun's position in azimuth changes through 90°, and the early and late birds would be expected to depart to the east and west instead of to the south, *if* they had learnt to fly at a fixed angle to the sun. Fig. 4 makes it clear that no such effect was obtained. Only two birds were within 30° of the fixed-sun-time direction, and these can be considered as the limits of scatter about the true home line. Comparing overall performances we have:

No. birds	Orientation time	Within 30° of home	Homing speed	2nd day	Stragglers
22 early and late	4'7	10	30'3	4	—
23 normal	5'4	13	31'3	1	1

The radical alteration in time of release may therefore have occasioned some confusion, but without directional significance. If these birds were using the sun to find the home/training direction, they must be able to appreciate and allow for its movement across the sky, without having witnessed that movement.

There remained the possibility that pigeons might fly at a fixed angle to the sun at an early stage of their training. This was tested in the course of releases off the training line which are discussed later (see Fig. 6). In Test 12, ten pigeons that had only flown twice from 25 miles were released 6-7 hr. later than their individual release time in Test 11. None vanished within 30° of the fixed-sun-time line.

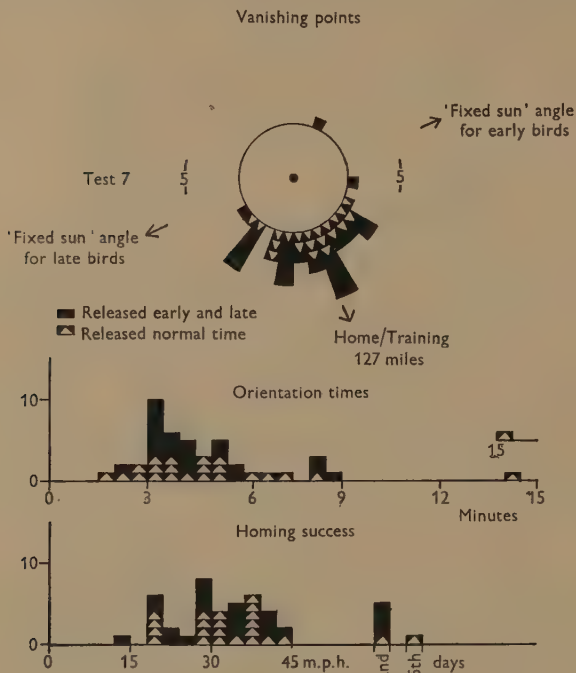


FIG. 4. Absence of a fixed sun-time-direction relation.

A further ten were used in Test 15, having flown 25 miles twice and 53 miles twice on the training line. The maximum deviation from the individual mean time of release in Tests 11, 13, 14 varied from 2 to 10 min. In Test 15 they were released between 5 and 7 hr. later. Two of these vanished within 30° of the fixed-sun-time line, but no significance can be attached to this as the general departure 'fan' was wide.

These results strongly suggest that at no stage in their training do young pigeons learn to fly at a *fixed* angle to the sun's position in azimuth.

BEHAVIOUR OF PIGEONS WHEN RELEASED OFF THE TRAINING LINE

There are many instances known in which pigeons have homed successfully from directions other than that of training, but these were generally accidental or the result of emergencies. Thus in Test 3 one bird arrived with a 'foreign' group at

their loft in Birmingham. Released there at 18.50 the next day, it reached its home, 90 miles away in a novel direction, before 0730 the next morning. A few experiments have been reported that suggested that pigeons were not entirely dependent on the training line (Thauzies, 1909; Riviere, 1929; Gibault, 1933; Heinroth, 1941). The data provided was limited or otherwise unsatisfactory however,

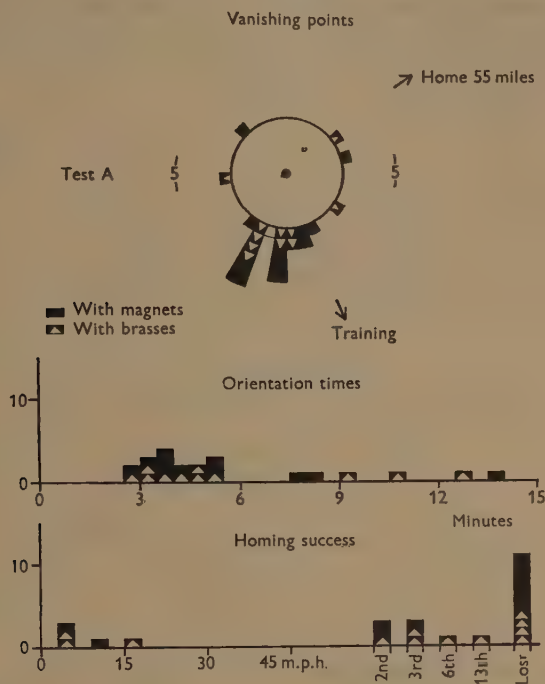


FIG. 5. Partially trained pigeons: orientation in training direction. Absence of effect by magnets.

and had little weight when compared with the heavy emphasis on one-direction flights in pigeon racing. A series of tests have therefore been carried out to determine whether pigeons, after varying amounts of training, could distinguish the home direction from the training direction when the two were no longer coincident.

In Test A, twenty-four pigeons that had had a minimum of training—grouped releases up to 16 and 18 miles north and north-west of the loft—were released singly 55 miles south-west, the home line now making an angle of 107° with the training line. The results (see Fig. 5) were:

No. birds	Orientation time	% Within 30° of		Homing speed	2nd day (%)	Stragglers (%)	Lost (%)
		Training	Home				
24	5.8	39	9	9.2	12	21	46

These birds showed a strong tendency to start out in the training direction, resulting in slow, straggling returns and the loss of nearly half their number. Two did start out in the home direction, both returned, one giving the highest speed of the test. Eight of the survivors were released again at the same point (Test B). The training line was abandoned, but no concentration in the home direction was observed. Homing success was, however, much improved, all save one returning on the day at an average speed of 21.7 m.p.h.

Four more tests were carried out with birds that had had more training, details of this and the results being tabulated below:

Test	No. birds	Previous training to N.N.W.	Release point	Angle between home/training lines
12	10	25, 25 miles	49 miles W.	079°
15	10	25, 25, 53, 53 miles	62 miles W.	082°
8	47	24, 24, 49, 49, 78, 78, 127 miles	79 miles W.S.W.	090°
9	33	Ditto and test 8	69 miles S.S.E.	175°

The relation of release points to the training line can be seen from Fig. 1. All releases were in sunny conditions with well-broken cloud. Results:

Test	Orientation time	Within 30° of		Homing speed	2nd day (%)	Stragglers (%)	Lost (%)
		Training (%)	Home (%)				
12	5.8	20	40	22.6	30	Nil	30
15	4.1	10	30	26.9	30	10	20
8	3.3	23	30	23.2	25	9	13
9	4.5	Nil	35	17.7	21	3	6

These are expressed in histogram form in Figs. 6, 7 and 8.

These birds showed a stronger tendency to start in the home direction than in the training direction, the homeward orientation of the departure 'fan' being very clear in the last two tests. That shown in Fig. 8 is a striking testimonial to the almost complete abandonment of the training line. This is in complete contradistinction to the results of Test A. There were also fewer losses and faster returns. Before the implications of these results can be fully discussed it is very necessary to see whether there is any likelihood of the birds having had previous experience of the release points.

Pigeons on exercise are rarely absent for more than an hour or two, all of which may not be spent in flight. As they prefer to fly in groups it is unlikely that odd birds could slip away without being noticed. These groups are sometimes identified up to 10 miles from the loft, but again may be seen passing over the same locality several times. It is most unlikely that systematic probing in all directions is carried out. Very rarely young pigeons are seized with an unexplained panic and refuse to return to the loft for several hours. There was one such occurrence with the Duxford

birds in 1949 (twenty-two used in Test 8 and fourteen in Test 9). Although they were out for a total of 7 hr. we can state with fair certainty that they did not go much more than 20 miles from the loft. This is based on resightings in the neighbourhood, and by odd birds dropping from the flock into 'foreign' lofts. It is quite certain that they could not have reached either of the release points for Tests 8 and 9. It is therefore probable that young pigeons will become familiar with country round the loft to a maximum radius of 20 miles only.

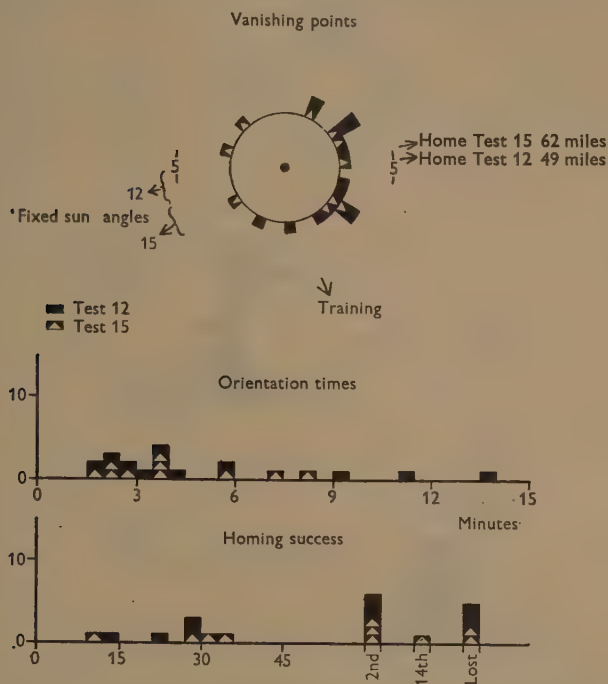


FIG. 6. Partially trained pigeons: orientation predominantly homewards. Absence of fixed sun-time-direction relation.

If the one-direction training had succeeded in its purpose the majority of the pigeons should have had no experience of country far to the west and south of the training line. In particular, birds that returned at high speeds could have deviated little from the straight training line. Those that in any one test did not return on the day might possibly have reached the critical release points. In Tests 12 and 15 only one bird was in this category, and this was lost on the critical test. The birds in Test 8 may similarly be divided into two categories. The second was extended to include birds that on any one test had been absent for longer than the time required to fly at 30 m.p.h. from the release point in question to the release point for Test 8

and on to the loft. In the same way birds in Test 9 may be divided into two categories to which the release point might respectively be considered unknown ('U') and possibly known ('K'). The division is here based on the performances in test 8, since it is from this release that stragglers were more likely to have reached the southern point. Comparing the performances of the two categories we have:

Test	No. birds	Orientation time	Within 30° of home (%)	Homing speed	2nd day (%)	Stragglers (%)	Lost (%)
8	23 'U'	3.1	35	24.8	22	9	9
	24 'K'	3.5	25	21.3	29	8	17
9	20 'U'	4.0	53	19.3	5	5	5
	13 'K'	5.3	8	12.9	46	Nil	8

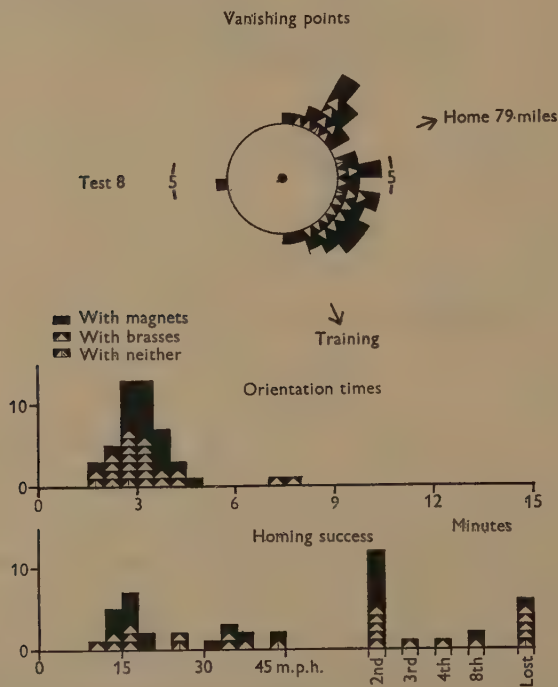


FIG. 7. Trained pigeons: orientation predominantly homewards. Absence of effect by magnets.

In both tests those birds that might possibly have had previous experience of the release point did considerably poorer in all respects than those which could not have had such experience, i.e. no question of knowledge of the surroundings can be invoked to explain the results.

It can therefore be stated that when young pigeons in various stages of training were released in unknown areas off the training line they showed a pronounced homeward orientation. This constitutes, perhaps, the most concrete evidence yet brought forward for the existence in birds of some form of true navigation in two co-ordinates.

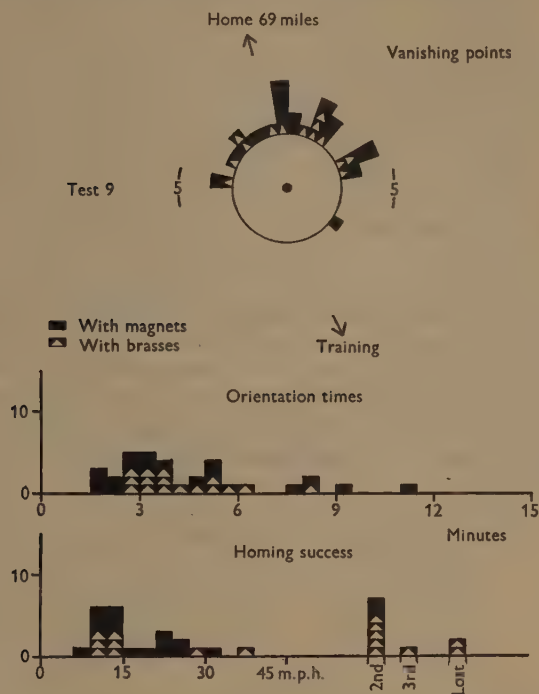


FIG. 8. Homeward orientation by trained pigeons. Absence of effect by magnets.

INDIVIDUAL VARIATION IN NAVIGATIONAL ABILITY

It has been shown that pigeons with blameless training histories put up the better performances off the training line, and this suggests that individual variation in navigational ability exists. If certain birds were superior performers in all respects it would be expected that they would do well no matter in which direction they were taken. The performance of thirty birds that took part in all the three major tests of 1949, Tests 7, 8 and 9, can be examined from this point of view. On the basis of their homing success in the last two tests they may be divided into three subequal categories:

'Good': day returns at high speed (av. over 24 m.p.h.)—9 birds.

'Moderate': day returns at low speed (av. below 24 m.p.h.)—8 birds.

'Poor': returned 2nd day or later in Test 8 and/or 9—13 birds.

Overall performances in the three main tests were:

	Orientation time			Within 30° of home (%)		
	Test 7	Test 8	Test 9	Test 7	Test 8	Test 9
Good	4.1	3.0	4.1	78	55	63
Moderate	4.2	3.2	4.1	63	12	25
Poor	6.1	3.4	5.7	60	23	17

	Homing speed		
	Test 7	Test 8	Test 9
Good	35.5	31.3	25.1
Moderate	28.2 and 2 on 2nd	15.9	11.1
Poor	25.7	13.3 and 7 on 2nd, 1 on 3rd	14.6 and 7 on 2nd, 2 lost

The nine 'good' birds gave better performances in *all* respects than the rest when released in unknown areas on an extension of the training line, at right angles to it or in the opposite direction. Again, in most respects the 'moderate' birds were consistently better than the 'poor'. The comparison cannot be carried back in detail to earlier releases at unknown points owing to insufficient data. The results obtained with these birds in Tests 3 and 5 may, however, be combined:

	Sorties	Orientation time	Within 30° of home (%)	Homing speed
Good	14	4.6	50	19.0 and 1 on 3rd and 4th
Moderate	14	4.1	36	18.6 and 1 on 2nd
Poor	21	4.8	45	19.9 and 1 on 2nd and 4th

In these earlier tests therefore no great differences are shown, nor would they be expected, as any special navigational ability would be of less importance on shorter flights over the training line. It is of interest to note, however, that only 14 % of the good sorties started out more than 45° off the home line, as opposed to 53 % of the other two groups.

When thirteen birds not used in all three major tests are classified rather arbitrarily on their partial performances, we find that of the sixty pigeons used in 1949, 18 % were 'good', 15 % 'moderate' and 38 % 'poor', the rest being lost. This emphasizes the small proportion of pigeons that can be expected to become able navigators. The important conclusion is thus reached that there is undoubtedly a wide variation in individual navigational ability among pigeons that have received identical treatment. This indicates that the problems of bird navigation are unlikely to be solved by mass experiments that cannot distinguish individual performance from the effects of chance. Also solitary records of outstanding performances cannot be ignored. It complicates the experimental technique required, especially in the case of wild

birds, and it may be that, for the present, work with pigeons is the only practical approach. It suggests an explanation for the conflicting results obtained in this field. In particular, the very different results obtained in Test A as compared with the other releases off the training line can be examined. Either the different lots of birds had equal potentialities but had developed them to different degrees, or they may have inherently possessed unequal potentialities.

The small increase of known territory prior to Test 12, as compared to Test A, would only benefit birds flying in roughly the home direction. This applies to a lesser extent to the other releases off the training line. It is possible, however, that there is a maturation of some navigational faculty during training, other than that of flying in the training direction. There is plenty of evidence that young pigeons that have received no training will fail to home from distances easily flown by trained birds of the same age. A few returns of untrained birds have been reported, but as they stand they could be attributed to chance. The present results indicate that if maturation through training occurs, it is very rapid during the early stages (e.g. between the equivalents of Test A and Test 12) and relatively slow thereafter. Possibly continuous training in one direction may actually blunt the development of any more advanced navigational faculties.

Another possibility is that some innate faculty develops independently of training, by a natural maturation process. The majority of the birds used in Test 12 were of the same age as those in Test A, but we may compare the performances of birds from two breeding 'rounds' of the same stock used on the same tests after the same training. No consistent differences in orientation behaviour were found; in particular, there was no tendency for the older birds to show stronger homeward orientation. On the training line the homing success of the two rounds was very similar, losses being equal, e.g. for Duxford stock:

Round	Age at first single release	1949		1950	
		Used	Lost (%)	Used	Lost (%)
I	20, 19 weeks	13	23	27	41
II	15, 14 weeks	19	26	28	36

In the case of the critical western releases, however, the round II birds had higher losses:

Test	Round	Age at test (weeks)	Used	Lost (%)	Stock
A	I	18	18	33	Easton
	II	13	6	83	Easton
12 and 15	I	20, 22	6	17	Duxford
	II	15, 17	8	50	Duxford
8	I	25	9	11	Duxford
	II	21	13	23	Duxford
20 (see p. 530)	I	24	10	10	Duxford
	II	20	9	66	Duxford

No other differences in the performances of the two stocks is to be discerned, the Duxford survivors in 1949 being classified:

Round	Good	Moderate	Poor
I	2	3	4
II	1	3	6

It is probable therefore that the difference between the two age groups is one of stamina, upon which heavy calls would be made on the critical releases, and is not due to any differential maturing of the navigational faculty.

There remains the possibility that the birds were of unequal inherent potentialities. There was no selection of the birds used in critical tests. In Tests 12 and 15 the birds were taken at random, in the other tests the whole available stock was used. Any natural selection of bad homers by losses on the training line was similar in all cases, amounting to 20 % before Test A, 12 % before Test 15 and 22 % before Test 8. The suggestion is therefore that the Easton stock contained a smaller proportion of potentially able navigators. This cannot be checked directly, as the Easton birds were not subjected to full classification trials. We can, however, compare the performances of first round birds from two different stocks, Duxford (13) and Sawston (21), in 1949. These were subjected to the same tests under the same conditions throughout. Three Duxford birds (23 %) were lost on the training line against one Sawston (5 %). Both stocks lost one bird on Test 8. The survivors were classified:

Stock	Good	Moderate	Poor
Duxford	2 (15 %)	3	4
Sawston	7 (33 %)	3	9

These are indications that the Sawston stock contained proportionately more able navigators. It is therefore possible that the Easton stock contained even less than the Duxford stock, and so produced the observed difference in results. It would seem that the less able navigators can learn to fly in the training direction, but do not develop the more subtle faculty that permits them to determine the homeward direction. Insufficient controlled data are yet available to make a detailed analysis of pigeon-breeding records a rewarding one. It is of interest to note that ten of the birds classed as 'good' had sibs of the same age. Two were classed as 'moderate', five 'poor' and three were lost.

The probability that different stocks of wild birds may also contain different proportions of able navigators is an interesting speculation that cannot be developed further in this paper.

INVESTIGATION OF THE POSSIBLE FACTORS CONCERNED IN THE ORIENTATION OF PIGEONS IN THE HOMEWARD DIRECTION

It has been shown that at least a proportion of pigeons are capable of orientating themselves in the general direction of home, whether or not this coincides with the line on which they have been trained. They are further able to maintain this general

direction over long stretches of unknown country. Such achievements clearly require a different order of navigational ability to that for flying in one particular direction only. Many theories have been put forward, and some have been examined experimentally in the course of the present work.

Any idea of random scatter from the release point may be excluded, likewise a spiralling out therefrom. Random search for known landmarks may also be discounted, for though a certain amount of search is carried out, it is largely confined to sideways sweeps about a general line of flight. While it is possible that air masses and wind directions would be of some help if they remained very constant, the rapidly changing weather conditions over England preclude such assistance in the present experiments. The pigeons homed successfully with head, beam and tail winds. Sensitivity to any of the effects produced by the earth's rotation may be discounted on theoretical grounds. Vague theories of 'space sense' and telepathy need not concern us here. The theory of a sensitivity to the earth's magnetic field has been resurrected by Yeagley (1948) and may be considered in more detail.

The original idea of some form of direct sensitivity has been replaced by one of indirect sensitivity, requiring the detection of minute electrical changes in the bird's body produced by its passage through the earth's field. The somewhat regular variation in the latter would then provide an indication in one co-ordinate of the relative positions of release point and home. There are strong theoretical objections to this modified theory which have been discussed in detail by Wilkinson (1948). Yeagley attached magnets to the wings of pigeons, thereby superimposing a pulsating field. He claimed that the performance of ten such birds was considerably worse than that of ten controls. The difference was not statistically significant and the loss in flight of several magnets suggests that they may have been unskilfully attached (sewn through the metacarpal joints) while brass control bars were put on by one used to such work. On this inadequate basis Yeagley embarked on a massive series of experiments, using hundreds of pigeons, which gave results he interpreted as showing that they navigated by means of a grid, one co-ordinate of which was provided by the detection of the vertical component of the earth's magnetic field, the other by detection of the Coriolis forces consequent on the earth's rotation. The planning of the experiments left much to be desired, observational bias was not prevented and the method of interpretation was faulty. The various experimental flaws that invalidate Yeagley's claims have been pointed out by Thorpe (1948). The 'magnetic wing' experiment was repeated by Gordon (1948) with entirely negative results. However, it was later found (Griffin in litt. 1949) that at least some of the pigeons had been previously used over the test course, and this throws doubt on the whole experiment. The theory was further tested on three of the critical releases off the training line in the present series.

Two methods of imposing a fluctuating field near the bird were used. In Test A (p. 517, Fig. 5) 4 g. steel magnets of 40 by 4 mm. cross-section and magnetic moment 100 gauss/cm.³ were hung round the birds' necks and in flight differential accelerations caused them to swing with an amplitude of at least 30°. A fluctuating voltage was thus induced in the head region of approximately 0.30 μ V./cm., six times the

minimum required to confuse measurement of the 'natural' induced voltage if we require a navigational accuracy of 25 miles. Similar brass rods were attached to the control birds, all being put into position the evening prior to release. In Test 8 (p. 520, Fig. 7) and Test 9 (p. 521, Fig. 8) Alnico magnets 20 by 3 mm. cross-section, weight $1\frac{1}{2}$ mg., magnetic moment 100 gauss/cm.³, were used. One was stuck with Bostik along the quill of the outer primary of each wing at the base of the barbs. Similar brass bars were mounted on controls. On the outspread wing the magnets were about 15 cm. from the mid-line, and with a wing beat frequency of 3/sec. and an amplitude of 7 cm. they would induce a fluctuating voltage varying between 0.16 and 0.11 μ V. in the head region. Attachments were made 6 days before Test 8, and the birds given two 10-mile group flights from the training direction and also daily exercise. This accustomed them to flight with the additional weights without requiring them to exercise any special navigational faculties. No attachments were lost during the tests. Birds were released singly, two magnet releases

Test	Total	Orientation time	Within 30° of home	Homing speed	2nd day	Stragglers	Lost
A	12 magnet	5.7	1	8.4	2	1	7
	12 brass	5.9	1	9.7	1	4	4
8	22 magnet	3.3	9	22.5	7	1	1
	22 brass	3.4	5	21.9	5	3	4
9	17 magnet	4.6	8	17.8	2	Nil	Nil
	16 brass	4.5	3	17.3	3	1	2

being followed by two control releases giving the above results. There is no evidence at all that the magnets had any influence on the ability to orientate in the homeward direction and return home when released off the training line. In Test A, where the majority of birds orientated in the training direction, the magnets were also without effect, six birds with magnets vanishing within 30° of the training line as against three controls. We may therefore conclude that the detection and measurement of the earth's magnetic field plays no part in the simple or advanced types of navigation that have been demonstrated in the present tests. As the results are so entirely conclusive it is to be hoped that this theory will be finally discarded.

Generally connected with the magnetic theory have been many reports of birds disorientated by local electrical disturbances produced by thunderstorms, radio transmissions or radar. It is possible that such disturbances could, in certain circumstances, be detected by birds, but this would not necessarily prove that navigation was affected. As a test of any gross effects of radar, fifteen pigeons were released singly at R.A.F. Station, Waterbeach, Cambridgeshire, by kind permission of the Commanding Officer. The release (Test 19) was *in known country*, and the birds were subjected to 3 and 10 cm. beams from G.C.A. apparatus. Each bird was minutely observed through powerful glasses, but no change in demeanour was seen when entering or leaving the 3 cm. beam, nor when momentarily hit or followed continuously by the 10 cm. beam. There were no unusual evolutions nor any abnormal delay in departure, orientation time averaging 4.2 min. Pigeons held, or standing within a foot of the 3 cm. aerials, gave no reaction. A swallow flew steadily

and unconcernedly across the path of both beams. Such a test is not, of course, entirely conclusive, but if any organ or faculty is to be thrown out of gear by radar transmissions there should have been some indication of this even if the bird was not actively using them.

The theory of displacement recording by time/acceleration measurement is also of respectable antiquity. The crude version requiring the outward path to be retraced is logically unsatisfactory, and the circumstances of Tests 12 and 15 form an experimental test. The birds were taken along two sides of a triangle, the apex of that in Test 12 being the release point for Test 13, and in Test 15 that for Test 16 (see Fig. 1). Seven of the birds homed at speeds that showed that they must have completed the third side of the triangle. The more refined version requiring the maintenance of a constant appreciation of the home direction by a process of triangulation is theoretically most unlikely to be within the power of the organs at an animal's disposal. A number of attempts have been made to disrupt the normal function of the proposed mechanism (especially of the semi-circular canals). However, critical examination of the evidence shows that in all cases the possibility of birds homing by sight and search alone has not been excluded. Further tests were therefore made in the present series.

Pigeons were carried to the release points in a large lightproof drum turning horizontally at four revolutions a minute. The drum being slightly unstable, changes in speed and direction of the transporting vehicle caused it to press on its bearings and produce a momentary slowing. The outward journey through space of the individual birds was thus remarkably complicated by an irregularly varying rotation, about 1200 revolutions on the longest journey. In Test 18 seventeen birds were released 130 miles north-north-west (after training up to 87 miles north-north-west) following rotation on the outward journey of 4 hr. 55 min. Eighteen birds were carried in the usual covered baskets to act as controls. The rotated birds included all those that had been used in Tests 12 and 15, and thus had less experience of the training line. In Test 20 the birds were released 81 miles from the loft at right angles to the training line, sixteen being rotated for 4 hr. 10 min. and fourteen taken as controls. The latter included all the birds that had been rotated previously, i.e. none of the rotated birds in Test 20 had any previous experience of western releases. Lastly, in Test 21 the survivors were released 68 miles in the opposite direction to that of training, eight being rotated for 3 hr. 50 min. and eight taken as controls. In all cases birds were released singly, two rotated being followed by two controls. The results for these three tests are given below (see also Figs. 9, 10):

Test	Total	Orientation time	Within 30° of home	Homing speed	2nd day	Stragglers	Lost
18	17 rotated	4.5	8	20.9	2	3	Nil
	18 controls	5.1	10	20.1	2	1	2
20	16 rotated	6.6	5	15.7	2	3	5
	14 controls	6.8	2	19.5	4	3	4
21	8 rotated	9.5	1	10.4	Nil	1	2
	8 controls	5.5	2	15.0	2	1	3

As no significant differences are to be discerned between the two categories, it can be said that rotation on the outward journey does not affect navigation from an unknown point on an extension of the training line or in radically different directions. It is therefore extremely unlikely that navigation in pigeons makes use of any time/acceleration mechanism. An absolutely final answer would require similar critical

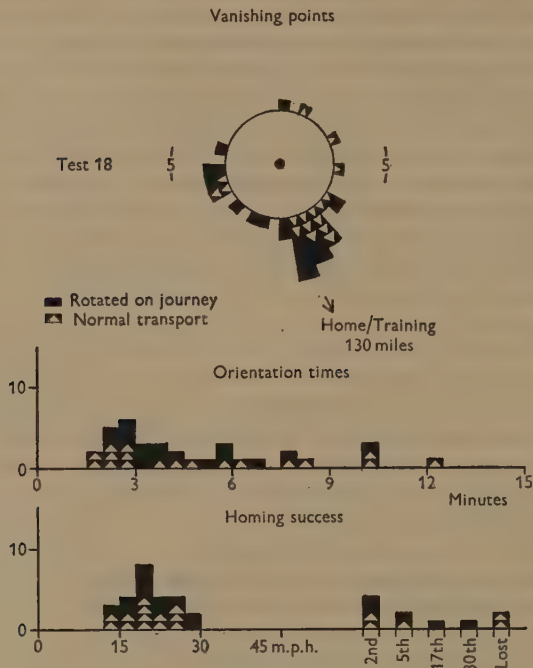


FIG. 9. Absence of effect by rotation on outward journey in release on training line.

releases of pigeons in which the probable receptors for acceleration measurement—the semicircular canals—had been destroyed. Such operative procedure is undesirable, as the effects may be more widespread than anticipated, and only negative results would be acceptable. Transport under heavy anaesthesia would be another test, again not absolutely final. The observation that most pigeons doze or sleep on the outward journeys is in itself an argument against a displacement-measuring mechanism.

We have seen (p. 514) that overcast skies had an adverse effect on orientation at a point on the training line, and the suggestion was that the pigeons were using the sun's position to determine the training direction. Test 7 (p. 516) showed that they must then be capable of taking large changes in azimuth into account and of at least ignoring large changes in altitude, and still be able to determine a particular direction.

This raises the query whether a pigeon could 'fix' its position by the classical method of mariners—latitude from the sun's altitude and longitude by time differences as observed from its position in azimuth. It is improbable that a pigeon could do this from a glimpse of the sun on any day of the year, as this would require a detailed knowledge of the apparent changes in the sun's position throughout the year. The observed general homeward orientation would, however, be explained if the birds

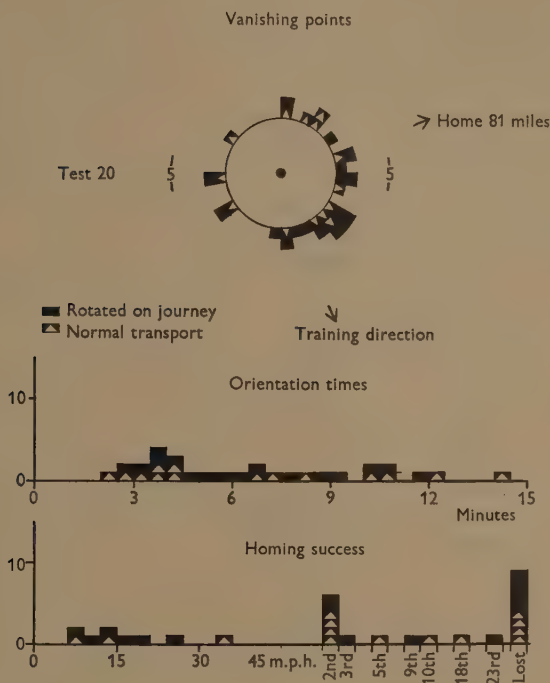


FIG. 10. Absence of effect by rotation on outward journey on release off training line.
(Effect of overcast see Fig. 11.)

could detect a gross difference between displacement north or south and east or west of the loft. To use the information available from the sun's position at the time of release (varying between 0530 and 1300 G.M.T.) the birds would be required to (1) estimate the highest point of the sun's arc; (2) measure the sun's altitude at that point and the time (according to some independent chronometer) at which it would be reached (alternatively, the angle in azimuth that the sun's observed position made from the estimated south point might be measured); (3) compare these measurements with those obtaining at the loft on, say, the previous day; (4) interpret the difference between the two sets of measurements.

The arc traced out by the sun and the speed at which it moves along that arc remains practically constant from day to day, though, of course, the amount of the arc above the horizon changes progressively. Given an intimate knowledge of these factors it would theoretically be possible to estimate the highest point, and when it would be reached, from an observation of the sun's movement over a part of the arc. But, as the birds were unable to see the sun before release, the time available is only the average orientation time, during which the sun would have moved only about two diameters along its arc. Further, with displacements from the loft of the present order, the maximum angular differences from home conditions would be less than four diameters, and time differences less than 8 min. Measurements would have to be accurate to well within these limits. There is little evidence of any independent time-keeping mechanism in birds, and none as to its accuracy, but some form of physiological chronometer cannot be ruled out of hand. It should be noted that there are no definite physical limitations in the organ concerned with angular measurements, the bird's eye having a high resolving power, being adapted for the detection of movement and suffering no inconvenience from glare.

Even if the necessary measurements could be made they would still have to be compared with those made previously, requiring a remarkable spatio-temporal memory. The interpretation of the differences would also be difficult to credit. By being trained to the north the birds previously had actual experience only of a decrease in altitude and a slight decrease in azimuth. The interpretation of a large decrease in azimuth in Test 8 and an increase in altitude in Test 9 would necessitate advanced reasoning on the part of the birds or inherited information to cope with all possible combinations. It may be noted, however, that learning and individual experience does not seem to play as great a part in pigeon navigation as might be thought. This is suggested by the general lack of continuous improvement in navigational ability with further training (p. 518), and by the results of preliminary laboratory experiments (Matthews, 1950) which have indicated that navigational ability is not positively correlated with the ability to learn simple problems using visual clues.

While this simplest use of the sun's position for pure navigation would seem to be improbable, it is important that the theory should be tested experimentally. Work on these lines has not proceeded very far as yet. It is difficult to obtain completely overcast conditions with suitable flying weather in summer, as anticyclonic conditions do not produce the well-known 'gloom' seen in winter. Cyclonic overcast is frequently broken and of short duration. However, the effects of overcast on two critical releases off the training line have now been observed. In Test 20 (Fig. 11) thirteen birds were released singly with a high thin overcast and 9/10th to 10/10th low thick cloud cover. A rainstorm now developed lasting an hour, seventeen birds then being released singly under 8/10th low thick cloud. In Test 21 (Fig. 12) sixteen birds were released under low overcast conditions, though while eight of these were being released the sun was very faintly visible through the momentarily lightened clouds. The results in these tests may be compared with those made at the same places in 1949, Test 8 and Test 9. In the former the sun was visible throughout the

releases, the only cloud being high thin stratus 5/10th to 8/10th, while there was brilliant sunshine with cloud never exceeding 3/10th in the latter.

Test	Conditions	Orientation time	Within 30° of (%)		Homing speed	1st day (%)	Total
			Training	Home			
20	Overcast	7.8	38	15	10.9	38	13
20	8/10th	5.7	41	29	24.4	24	17
8	Sun	3.3	23	30	23.2	53	47
21	Overcast	8.6	37	37	12.2	25	8
21	Overcast, sun visible as a disk	6.1	25	Nil	11.5	63	8
9	Sun	4.5	Nil	35	17.7	70	33

Test 20

Vanishing points

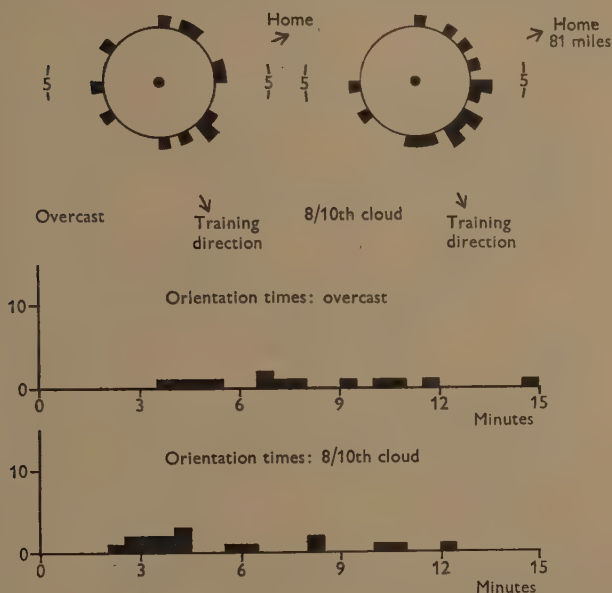


FIG. 11. Effect of overcast conditions on orientation in a release off the training line.

The comparisons certainly suggest that conditions of heavy cloud were having an adverse effect on orientation, though all the trends are not consistent. The increase of average orientation time with increasing cloud cover is noticeable in releases from both points. In Test 20 birds released under complete overcast returned slower, having shown poorer homeward orientation, than those released under 8/10th cloud. For Test 21 the few birds available does not make any strict comparison between the two groupings possible, but again were back on the first day when they had

been released with the sun visible as a disc. In both these tests there was a noticeably high proportion near the training line, which might be determined roughly from the sun's general position even when its co-ordinates could not be determined with accuracy. Only one bird started in this direction on both occasions, so there was no inability to fly in any direction other than this. Too much stress cannot be laid on homing success when comparing Test 20 with Test 8, as several heavy

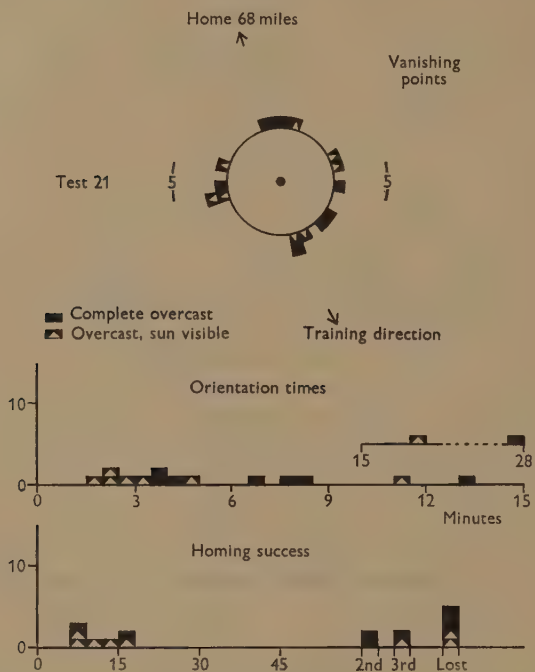


FIG. 12. Effect of overcast on release in opposite direction to training line.

thunderstorms developed across the homeward track accompanied by gale-force winds. For Test 21, however, flying conditions were excellent, and the overcast broke during the afternoon. There was a slight head wind instead of a tail wind as in Test 9, both of Force 2-3.

It is, of course, possible that the poorer showing in the 1950 tests was due to there being a smaller proportion of able navigators than in 1949. There can be no direct check of this, but it is unlikely to be more than a part of the answer. Thus a number of the birds used in Test 20 had previously homed well from releases off the training line (Tests 12 and 15). In the other major release, to the north, the performances in the two years was similar—compare Fig. 4 with Fig. 9; a rather poorer homing performance in 1950 being explained by bad weather conditions

which developed across the track. In any case there are still the differences within the tests themselves to be explained.

We have therefore at least a suggestion that overcast has a disorientating effect on pigeons released off the training line. Clearly more work is required to confirm or deny this, but as it is the only positive indication that has been obtained this should be well worth while. The requirements for rough sun navigation outlined above are also open to experimental investigation, particularly the necessity for an accurate physiological time sense and for a recent experience of sun conditions at the home loft. If it should be shown that pigeons are dependent on the sun for navigation this would fit in well with the fact that they will not home at night or in dense fog. It is true that much migration in wild birds takes place at night or beneath overcast, but if, as much contemporary work suggests, migration over unknown country is in a general *direction* only, the latter could have been determined at sunset or by a short glimpse of the sun respectively. In homing experiments with wild birds no returns have been made at such speeds as would have necessitated flying through the night.

RECORDING OF THE HOMEWARD TRACK IN PIGEONS

It would clearly be useful if we could have an accurate account of the homeward track, particularly when (as with wild birds) the overall speed is small and evidence is required as to how much exploration has taken place. However, the most interesting pigeons are those that home swiftly from various directions and can have deviated little from the straight line. Determination of the track components would still be of value, especially when assessing the efficacy of any method of disorientating the birds. Developments could also be applied to wild birds.

Griffin (1949 in litt). and Hitchcock (1950) have been successful in following flocks of pigeons by means of aircraft. The wide individual variation in navigational ability suggested by the present work makes it doubtful whether the tracking of flocks would produce results of sufficient interest. Accordingly, attempts have been made to follow individual pigeons and small groups, by the courtesy of the Officer Commanding, Cambridge University Air Squadron, and with the enthusiastic co-operation of his officers. Three types of aircraft were tried, the most useful being an Auster Mark VI, which has a low stalling speed, good all round visibility and carries two observers. That available lacked both intercommunication and wireless contact with the ground. The trials involved three individual pigeons and fifteen groups of three birds. None of those available had much white in their plumage, and the light 'mealy' birds, otherwise the most visible, were quickly lost over ripening corn. Great difficulty was experienced in holding the birds in sight during their frequent sharp changes of course, and in the event no group was followed for more than 10 min.

Automatic recorders of the track, attached to the bird itself, would be most useful. A start has been made with the flight duration recorder designed by Wilkinson (1950). This was used in Test 8 in 1949 and on a larger scale in 1950. Attention has been directed to full trials of the apparatus itself, and the elimination of various

snags and inadequacies that were not at first apparent. The number of satisfactory flight records is limited and does not merit publication at present. General conclusions to date are that young pigeons will fly for up to 6 hr. on the day of release, those returning after a longer interval generally having been down for some of the time, though one continuous flight of $10\frac{1}{2}$ hr. is recorded. In the case of those returning on the second or subsequent days the average daily flight has been about 4 or 5 hr., even in one that returned on the 18th day.

REVIEW OF RESULTS, AND CONCLUSIONS

This series of experiments has shown that pigeons are capable not only of learning to fly in a fixed direction on release in unknown country, but of orientating in the homeward direction when released off the training line. The latter ability varies widely between individuals and probably has an inherited basis.

Several of the theories that have been put forward are too unsatisfactory theoretically to need further investigation. Others can be considered to have been eliminated by the present work, at least in the case of pigeons. They are: random or systematic search for landmarks, use of atmospheric clues, measurement and interpretation of the earth's magnetic field, and displacement recording by a time/acceleration mechanism. It has been shown, however, that overcast skies had an apparently disorientating effect on pigeons released on and off the training line. The use of the sun to obtain the training direction would be quite reasonable, though it has been shown that it is not by means of any constant sun-time angle. Suggestions have been put forward for a rough form of true navigation involving the measurement of the sun's position in two co-ordinates. This seems to be most unlikely but not wholly impossible. Much more work in this direction is clearly required.

The present research has thus by no means solved the problems of bird navigation. It has, however, shown that there *are* problems to be solved, and indicated a possible line towards their solution.

SUMMARY

1. A series of field experiments were carried out in 1948, 1949 and 1950 to establish the facts of navigation in homing pigeons and to begin an investigation of the factors involved.
2. A total of 158 pigeons aged 3-4 months were used in twenty-three tests involving a total of 902 sorties, all but 153 being by birds released singly.
3. Three indices of performance were used, giving the degree of uncertainty on release, the accuracy of the initial course and the success in regaining home.
4. It was shown that pigeons are able to fly in one particular direction when released at a series of unknown points at increasing distances north-north-west of the loft. The following of experienced birds or the use of distant landmarks were excluded.
5. Overcast skies had a disorientating effect on these training-line releases. It was shown that if the sun was being used to determine the training direction, it was not by any simple fixed-angle relationship to home. The birds could take large

changes of azimuth into account, and at least ignore changes in altitude, without having witnessed the sun's movement.

6. Releases were also carried out in unknown areas at right angles to or in the opposite direction from the training line. Some of the birds flew in the training direction and homed badly. But a good proportion showed a definite orientation in the home direction and returned with fair success.

7. This true navigational ability was shown to vary widely amongst individual pigeons. The proportion of able navigators also varied in different stocks. There is little evidence of a continuous maturation of the faculty with experience and none with age. There are suggestions that it is not connected with a learning process.

9. The established facts require more than a random or systematic search for landmarks, or the use of atmospheric clues as an explanation.

10. It is shown by experiment that pigeons do not depend on the interpretation of the earth's magnetic field to be able to orientate in the training or in the home direction.

11. Other experiments show that a displacement recording mechanism based on time/acceleration measurements is extremely unlikely.

12. The only positive lead obtained is a definite suggestion that overcast had a disorientating effect on pigeons released *off* the training line. This requires detailed confirmation. The simplest way in which a pigeon could obtain a rough estimate of its position relative to home by measuring the sun's co-ordinates is considered.

13. Attempts to follow individual pigeons or groups of three by aircraft have so far been unsuccessful. An automatic flight recorder has been used successfully to some extent.

I should like to acknowledge my outstanding debt to Dr W. H. Thorpe for his constant encouragement and facilitation of my work. Dr D. H. Wilkinson has been an invaluable guide in matters physical, especially magnetic problems, and actively helped in several field tests. The experiments would have been impossible without the part played by Messrs P. Cope (and his loftman, S. Small), L. R. Duke and L. A. Meredith in rearing the birds and undertaking their preliminary training. For practical advice and assistance I am indebted to Drs R. H. J. Brown and M. G. M. Pryor. I am most grateful for the stimulation of personal discussion of this research with Prof. J. Gray, Drs H. B. Cott, R. G. Newton, A. Landsborough Thomson and Messrs W. B. Alexander and R. E. Moreau. Similarly, I have benefited from correspondence with Profs. D. R. Griffin, H. B. Hitchcock and V. C. Wynne-Edwards. The work was part of a programme of research carried out while I was in receipt of a Maintenance Grant from the Ministry of Education. Part of the experimental expenses in 1949, and all those in 1950, were met by a grant from the Royal Society. I should like to express my deep gratitude to both these bodies.

REFERENCES

- GIBALT, G. (1930). Recherches sur l'orientation du pigeon voyageur. *C.R. Congr. Ass. Avian. Sci.* pp. 250-2.
- GIBALT, G. (1933). Recherches sur l'orientation du pigeon voyageur. *Alauda*, **5**, 5-26.
- GORDON, D. A. (1948). Sensitivity of the homing pigeon to the magnetic field of the earth. *Science*, **108**, 710-11.
- GRIFFIN, D. R. (1944). The sensory basis of bird navigation. *Quart. Rev. Biol.* **19**, 21-32.
- GRUNDLACH, R. H. (1932). A field study of homing pigeons. *J. Comp. Psychol.* **13**, 397-402.
- HEINROTH, O. & K. (1941). Das Heimfinde-Vermogen der Brieftauben. *J. Ornith.* **89**, 213-56.
- HITCHCOCK, H. B. (1950). Aerial observation of homing pigeons. Amer. Soc. Zool. Meeting, 30 Dec.
- KNIERIEM, H. (1943). Voraussetzungen für schnelles Heimfinden der Brieftauben bei geringen Verlusten auf den Reisen. *Z. Tierpsychol.* **5**, 131-52.
- MATTHEWS, G. V. T. (1950). Ph.D. Thesis, Cambridge.
- NICOL, J. A. C. (1945). The homing ability of the carrier pigeon. Its value in warfare. *Auk*, **62**, 286-98.
- PLATT, C. S. & DARE, R. S. (1945). The homing instinct in pigeons. *Science*, **101**, 439-40.
- RIVIERE, B. B. (1923). Homing pigeons and pigeon racing. *Brit. Birds*, **17**, 118-38.
- RIVIERE, B. B. (1929). The 'homing faculty' in pigeons. *Verh. 6th Ornith. Kongr.* (1926), pp. 535-55.
- SCHNIEDER, G. H. (1906). Die Orientierung der Brieftauben. *Z. Psychol. Physiol. Sinnesorg.* **40**, 252-79.
- THAUZIES, A. (1909). Lacher de pigeons. *P.V. 6me Int. Congr. Psychol.* p. 834.
- THORPE, W. H. (1948). Recent biological evidence for the methods of bird orientation. *Proc. Linn. Soc. Lond.* **160**, 85-94.
- WILKINSON, D. H. (1948). Some physical principles of bird orientation. *Proc. Linn. Soc. Lond.* **160**, 94-9.
- WILKINSON, D. H. (1950). Flight recorders. A technique for the study of bird navigation. *J. Exp. Biol.* **27**, 192-8.
- YEAGLEY, H. L. (1948). A preliminary study of a physical basis of bird navigation. *J. Appl. Phys.* **18**, 1035-63.

THE ROLE OF RADIATION IN PHOTODYNAMIC ACTION

By G. CALCUTT

Department of Cancer Research, Mount Vernon Hospital and the Radium Institute, Northwood, Middlesex, England

(Received 3 April 1951)

(With Two Text-figures)

The literature concerned with photodynamic action is surprisingly reticent upon the subject of the role played by the radiation used to induce the effect. It is tacitly assumed that the radiation plays its part solely via the so-called 'sensitizing' agent. The possibility of direct action upon the test material appears to have been ignored. Recently (Calcutt, 1950), evidence has been put forward indicating that wavelengths greater than 3300 Å. are effective in influencing the intracellular availability of sulphhydryl groups. Additionally, it has been suggested that functional —SH groups are involved in the mechanism of photodynamic action (Calcutt, 1948). Further experimental work has been carried out to endeavour to elucidate this joint question of the role of radiation and —SH groups in photodynamic action.

MATERIALS AND METHODS

All the experimental work reported in this paper was carried out with thick cultures of *Paramecium bursaria* as the biological indicator. Irradiation was from a mercury-vapour lamp screened with a glass filter opaque to radiations of less than 3300 Å. The experimental work was done in a darkened room; examination of cultures being done with the aid of a dull light screened by a Wratten IIA filter. Exposures of cultures were made in small flat watch-glasses, each containing 5 c.c. of mixed culture and test solution. The end-point for each experiment was taken as that time when an estimated 90 % of the *Paramecia* concerned had been rendered motionless. As photosensitizers, 3:4-benzopyrene, fluorescein and quinine sulphate were employed; the first mentioned, as a colloid, and the other two as solutions, in tap water.

In a first series of experiments, measured samples of the *paramecium* cultures were irradiated under the lamp for various time periods immediately prior to the addition of the photosensitizer and return under the lamp. The times of irradiation then necessary for an estimated 90 % of the culture to have been rendered motionless were recorded. In a second series of experiments the cultures were irradiated as previously and then removed into total darkness for varying periods prior to adding the inhibitor and determining the time of further irradiation required for 90 % lethality. Controls for both series of experiments consisted of a number of exposures of cultures immediately subsequent to addition of the photosensitizer.

RESULTS

The first series of experiments showed conclusively that irradiation prior to the addition of the photosensitizer was effective in reducing the period of further irradiation required to achieve an end-point. Results are shown diagrammatically

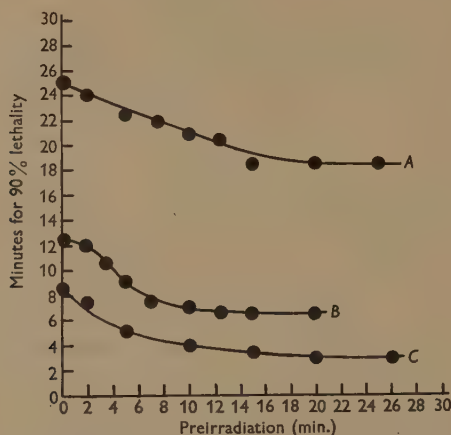


Fig. 1. The effects of pre-irradiation on photodynamic activity. Curve *A*, fluorescein, 1/4000; curve *B*, 3:4-benzpyrene, 1/150,000; curve *C*, quinine sulphate, 1/60,000.

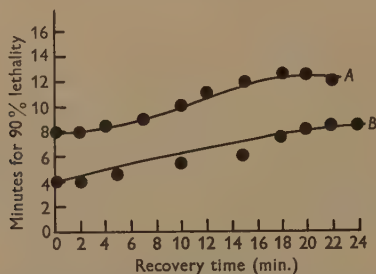


Fig. 2. Recovery from pre-irradiation. Curve *A*, 3:4-benzpyrene, 1/150,000, 7 min. pre-irradiation. Non-irradiated control: 12½ min. (mean of five readings). Curve *B*, quinine sulphate, 1/60,000, 10 min. pre-irradiation. Non-irradiated control: 8½ min. (mean of five readings).

in Fig. 1. These curves also demonstrate how the effect is increased up to a certain level as the period of pre-irradiation is lengthened. The flattening of the curves into a horizontal line indicates some limitation of the effect induced by the pre-irradiation.

For the second series, periods of pre-irradiation were arbitrarily selected by reference to Fig. 1. Using these fixed periods of pre-treatment, rest periods of varying lengths were employed, and it was found that recovery to the normal state occurred. This is illustrated by the specimen curves shown in Fig. 2. In the case of

the 3:4-benzpyrene the recovery phase appeared to be more rapid in the early stages than later, whereas with the quinine sulphate the process appeared to take place at a steady smooth rate. This apparent difference may be associated with the fact that the benzpyrene was colloidal and the quinine sulphate was in the more readily accessible form of a solution.

DISCUSSION

From the results recorded above it is apparent that the radiation has an effect upon the test material—in this case, *paramecium*—itself, without the intervention of the photosensitizer. Equally, this effect appears to be reversible. The possibility of the action being due to the presence of an unrecognized photosensitizing agent in either the culture medium or the cells themselves must be discounted, since the effect in question only reaches a certain level of intensity and then remains constant. If it was due to a photosensitizing agent it would be expected to be continuous until such a time as death of the cells ensued. It appears then that the increased sensitivity of the *paramecium* to the photo-sensitizing action after pre-irradiation is due to a cellular change which can only have been initiated by the radiation itself. Since identical radiation is used to elicit the photodynamic response it is to be expected that this cellular effect will occur during the process of killing by photodynamic action. It follows therefore that the irradiation plays a part in photodynamic action greater than that assumed by the view which may be summarized as: photodynamic action depends upon energy transfer via the photosensitizing agent.

Some evidence as to the mechanism of the action of the pre-irradiation treatment has already been offered (Calcutt, 1950). It appears from this that the radiation increases the availability of —SH groups, and since it also increases the photodynamic response this must be regarded as further evidence implicating sulphhydryl groups in the mechanism of photodynamic action. In this connexion it must be recalled that a pH movement to the acid side—also recognized as causing an increased exposure of —SH groups—facilitates photodynamic action (Blum, 1941, p. 89). It appears, then, that the mechanism of photodynamic action is dependent—at least, in part—upon the availability of exposed —SH groups, and that the radiation plays a direct role in inducing the exposure of such groups. On this basis it may be further suggested that the ultimate cell death concerned in the action results from the oxidation of a sufficient number of functional —SH groups to cause complete functional derangement of the cell. The role of the photo-sensitizing agent under these conditions would be to accelerate oxidative processes. Support for such an event is given by the demonstration (Calcutt, 1949) that 3:4-benzpyrene—one of the most potent of photodynamic agents—is effective in inducing the oxidation of thiol groups. Furthermore, the necessity for oxygen for the photodynamic process would under these circumstances be explained by the intermediary formation of hydrogen peroxide during the oxidation of thiols (Schaels, 1938). Finally, this scheme would be well in keeping with the generally accepted viewpoint (cf. Blum, 1941, p. 79) that the major site of photodynamic action is the structural protein of the cell. It is just such a site where pH change or irradiation would be expected to influence the availability of —SH groups by unfolding of the protein chains.

The scheme put forward above must at the moment be regarded as tentative. Further experimental evidence is being sought and will be reported as soon as possible.

SUMMARY

It has been demonstrated that, within certain limits, the radiation of cultures of *Paramecium bursaria*, which had been kept in the dark, with wave-lengths greater than 3300 Å., facilitates a subsequently elicited photodynamic response. A period of darkness following the pre-irradiation allows of a return to the normal condition. The role of radiation in the mechanism of photodynamic action is discussed.

REFERENCES

- BLUM, H. F. (1941). *Photodynamic Action and Diseases Caused by Light*. New York: Reinhold.
CALCUTT, G. (1948). 26th Ann. Rep. British Empire Cancer Campaign, p. 90.
CALCUTT, G. (1949). 3:4-Benzpyrene as an —SH inhibitor. *Brit. J. Cancer*, 3, 306.
CALCUTT, G. (1950). A factor influencing the intracellular exposure of —SH groups. *Nature, Lond.*, 166, 443.
SCHALES, O. (1938). Empfindliche Methoden zum Nachweis von Wasserstoffperoxyd und seiner Entstehung bei einigen Dehydrierungs-Vorgängen. *Ber. dtsh. chem. Ges.* 447–61.

CELL NUMBER IN HAPLOID, DIPLOID AND POLYPLOID MOUSE EMBRYOS

By R. A. BEATTY AND M. FISCHBERG

*Genetics Laboratory, Animal Breeding and Genetics Research Organization,
Institute of Animal Genetics, Edinburgh*

(Received 9 April 1951)

(With One Text-figure)

CONTENTS

	PAGE
I. Introduction	541
II. Material and methods	542
III. Results	542
(a) Haploid egg	542
(b) Triploid eggs	544
(c) Tetraploid eggs	545
(d) Hexaploid eggs	545
(e) Detailed analyses of the sources of variation in the number of nuclei of triploid and tetraploid eggs	545
(f) Combination of results	547
IV. Discussion	549
V. Summary	551
References	551

I. INTRODUCTION

In previous papers we have been concerned chiefly with identifying heteroploidy in early embryonic stages of the mouse, determining the type of heteroploidy, and measuring the frequency of occurrence either after experimental treatments, or in silver-stock mice, some of whose embryos are spontaneously heteroploid (Beatty & Fischberg, 1949, 1951 *a, b*; Fischberg & Beatty, 1950 *a, b*, 1951 *a*). To avoid laborious routine, the eggs of each mouse were mounted as a squash preparation on one slide. This meant that particular eggs observed under the binocular microscope could not usually be recognized in the preparations, and little could be said about the exact stages of development. One observation could, however, be made safely on squash preparations—the numbers of nuclei could be counted. Nuclear numbers listed in previous papers, together with a roughly equal body of new data, have been brought together in the present paper, and a detailed comparison has been made of nuclear number (and therefore cell number) in haploid, diploid and polyploid mouse eggs from the early cleavage to the late blastula stage. In some plants and invertebrates, cell number in polyploids and diploids is about equal, but the polyploid cells are larger than diploid, and the organism is consequently of giant size. In Amphibia, also, the polyploid cells are larger, but body size does not differ, because the polyploid organism contains fewer cells than the diploid. The present results, with the early mouse embryo, contribute to an understanding of the relative cell number in haploid, diploid and polyploid mammal embryos.

II. MATERIAL AND METHODS

Mice (*Mus musculus*) of varying genetic constitution were used. In some eggs, heteroploidy occurred spontaneously. In others, it was induced experimentally. Sources of data are listed in the legend to Table 1. The spontaneous and experimental series have been pooled for all purposes, the results from each series being similar. In most mice, eggs were recovered $3\frac{1}{2}$ days after copulation. A few were recovered after $4\frac{1}{2}$ and $5\frac{1}{2}$ days. The procedure for obtaining eggs and for making squash preparations has already been described (Beatty & Fischberg, 1951*a*), and the nomenclature reviewed in the same paper is used here. Attention has been confined to polyploid eggs (which in our material means triploids, tetraploids and hexaploids) and to haploids. In accordance with common usage, the pre-implantation mouse embryo is sometimes referred to as an egg.

In considering differences in nuclear number between eggs of the same chromosome number, we find much variation even in eggs from the same mouse, due, for instance, to different rates of development of individual eggs. There is also a variation between different mice, due to differences in the average times of ovulation, in the average rate of development, and to observational uncertainty in that the time of fertilization, as judged by the time of formation of a vaginal plug, was subject to an (absolute) error of $\pm \frac{1}{4}$ of a day. It was thought that the best procedure would be to obtain for each mouse an $xn/2n$ ratio, which is defined as the ratio of the mean number of nuclei in any given type of heteroploid eggs of the mouse divided by the mean number of nuclei in the diploid eggs of the same mouse. Thus, a $3n/2n$ ratio is obtained for triploid eggs, a $4n/2n$ ratio for tetraploids, and so on. The diploid eggs serve, in fact, as an internal control in each mouse, against which the nuclear number of heteroploid eggs can be compared. (In the detailed analysis, as shown on pp. 545-7, the raw data were first transformed into natural logarithms, for the reasons stated, and the final estimate of the $xn/2n$ ratio was obtained by taking anti-logarithms. This may be termed the geometric $xn/2n$ ratio.)

Table 1 is a protocol of all data available up to 1 September 1950 in which there was at least one diploid and one polyploid (or one haploid) egg per mouse.

An attempt has been made to present the main body of the paper, so that it can be perused independently of the formal statistical treatment on pages 545-7.

III. RESULTS

(a) *Haploid egg*

The single haploid egg (in mouse SiL/107) had 32 nuclei, and was accompanied by two diploid eggs with 25 and 26 nuclei. The $1n/2n$ ratio is therefore 1.25. Little reliance can be placed on a ratio derived from only three observations, but the ratio is higher than any of the eight ratios for tetraploidy and hexaploidy, and (with four exceptions) is higher than any of the 47 ratios for triploidy.

Table 1. *Protocol of numbers of nuclei in haploid, diploid, triploid, tetraploid and hexaploid eggs*

Each of these fifty-four mice contained at least one haploid or polyploid egg, and at least one diploid egg. Counts of nuclei were made on squash preparations of eggs, the eggs of each mouse being mounted on one slide. Eggs were recovered $3\frac{1}{2}$ days after copulation except for five mice—serial numbers SiL/121–142 inclusive, recovered after $4\frac{1}{2}$ days, and mouse $5\frac{1}{2}$ SiL/3, recovered after $5\frac{1}{2}$ days. In the first eleven mice, heteroploidy was induced experimentally by hot or cold shock methods, the data being extracted from other papers (Beatty & Fischberg, 1951*b* and Fischberg & Beatty, 1951*a*). In the remaining forty-three mice, heteroploidy was spontaneous; data for the seventeen mice from serial number SiL/2 to LSi/50 inclusive are extracted from an earlier paper (Beatty & Fischberg 1951*a*) and data for the other twenty-six are from unpublished records.

Serial no. of mouse	Diploid eggs		Triploid eggs		Tetraploid eggs	
	Individual eggs	Mean	Individual eggs	Mean	Individual eggs	Mean
4^{II}	60, 51, 54, 43, 56	52.8	56	56.0	—	—
157	66, 16, 60, 31	43.2	28	28.0	—	—
180	62	62.0	40	40.0	—	—
197	57	57.0	20	20.0	—	—
200	31	31.0	43, 29, 29	23.7	—	—
236	47, 55, 55, 25, 30	42.4	27	27.0	—	—
281	18	18.0	37, 27	32.0	—	—
310	47, 50, 49, 43, 38	45.4	35	35.0	—	—
269	27, 59, 59	48.3	61	61.0	23, 31	27.0
272	80, 69, 78, 83	77.5	—	—	45, 47	46.0
362	28	28.0	—	—	15	15.0
V/87.1	61	61.0	43	43.0	—	—
V/87.2	21	21.0	41	41.0	—	—
V/87.4	27, 29, 50, 47	38.2	33	33.0	—	—
V/89.1	57, 45, 61, 75	59.5	57	57.0	—	—
V/94.3*	18, 33, 37	29.3	29	29.0	—	—
V/99.6	60	60.0	21	21.0	—	—
V/103.1	68, 57, 59, 56	60.0	32	32.0	—	—
V/103.7	23	23.0	24	24.0	—	—
V/112.5	44, 33	38.5	23	23.0	—	—
V/122.3	36, 37, 48, 62, 55, 52	48.3	35	35.0	—	—
V/124.1	42	42.0	27	27.0	—	—
V/124.5	39, 35	37.0	33	33.0	—	—
V/128.1	16, 28	22.0	12	12.0	—	—
V/129.2	32, 34	33.0	18	18.0	—	—
V/134.6	35	35.0	26	26.0	—	—
V/69.7	34	34.0	24	24.0	—	—
SiL/121	74	74.0	65	65.0	—	—
SiL/125	160	160.0	54, 102	78.0	—	—
SiL/131	160, 175, 160, 150	161.2	115	115.0	—	—
SiL/142	100	100.0	59	59.0	—	—
$5\frac{1}{2}$ SiL/3	166, 310, 340	272.0	310	310.0	—	—
SiL/2	45, 49	47.0	23	23.0	—	—
SiL/13	54, 46, 42, 58, 58, 49, 58, 50	51.9	30	30.0	—	—
SiL/31	92, 61, 78, 63	73.5	42, 56	49.0	—	—
SiL/37	67, 56, 32	51.7	—	—	50	50.0
SiL/43	33, 40, 31, 37, 35, 41	36.2	36	36.0	—	—
SiL/69	27, 54, 45, 62, 51, 22, 46, 52	44.9	20	20.0	—	—
SiL/75*	46, 49, 31, 43, 45, 57	45.2	—	—	—	—
SiL/83	41, 56, 39	45.3	35, 43	39.0	—	—
SiL/100	51, 43, 52, 46	48.0	32, 20	26.0	—	—
SiL/107*	25, 26	25.5	—	—	—	—
SiL/109	41, 26	33.5	30	30.0	—	—
SiL/113	47, 52, 51	50.0	33	33.0	—	—
SiSi/116	31, 31, 44, 35	35.2	33, 25	29.0	—	—
LSi/21	52, 33, 55, 31, 32	40.6	29	29.0	—	—
LSi/32	49, 40, 44, 30, 49, 32	40.7	36	36.0	—	—
LSi/47	36, 16, 29, 37, 28, 33, 31, 31	30.1	24	24.0	—	—
LSi/50	23, 29, 41, 39, 47, 45, 31, 26	35.1	—	—	11	11.0
D/13	53, 52, 42, 47	48.5	39	39.0	—	—
E/5	33, 44, 40	39.0	—	—	12	12.0
H/6	55, 53, 48, 53	52.2	54	54.0	—	—
J/19	27, 29	28.0	55	55.0	—	—
J/24	59, 56, 44	53.0	44	44.0	—	—

* V/94.3 contained also a hexaploid egg with 12 nuclei; SiL/75 a hexaploid egg with 4 nuclei; SiL/107 a haploid egg with 32 nuclei.

(b) *Triploid eggs*

The number of nuclei in triploid eggs, relative to the number in diploid eggs of the same age, may be expressed in several ways. In Table 1 there are forty-seven mice each with at least one diploid and at least one triploid egg. In each mouse, the $3n/2n$ ratio (the mean number of nuclei in the triploid eggs divided by the mean number in the diploid eggs) may be calculated. The result is summarized in Table 2,

Table 2. *Summary of $3n/2n$ ratios of cell number in eggs, calculated from the forty-seven mice in Table 1 which contained at least one triploid egg*

This is the arithmetic $3n/2n$ ratio, i.e. the mean number of cells in triploid eggs of a mouse, divided by the mean number of cells in diploid eggs of the same mouse.

Class of ratio	Frequency
0.3-	2
0.4-	3
0.5-	6
0.6-	7
0.7-	7
0.8-	9
0.9-	4
1.0-	4
1.1-	1
1.2-	1
1.7-	1
1.9-	2
Total	47

from which it may be seen that there is considerable variation, but the most frequent ratios (nearly half of them) lie in the groups 0.6-0.8. The average of the forty-seven ratios is 0.83. When the ratios are arranged in order of magnitude, the median ratio (the 24th from either end of the series) is 0.74. The sum of the forty-seven means for triploid eggs is 2033.7, the corresponding figure for diploids being 2567.7; the ratio of these two numbers is 0.79. Thus, however expressed, the number of nuclei in triploid eggs is about 0.7-0.8 the number in diploid eggs; i.e. there are fewer nuclei in triploid than in diploid eggs. Is this difference in nuclear number significant? If there were no real difference, the expectation would be $23\frac{1}{2}$ ratios above a value of 1.0 and $23\frac{1}{2}$ below 1.0. The actual figures were 9 ratios above 1.0 and 38 below 1.0. The chance of the observed distribution differing from the expected solely through sampling error is less than one in a thousand. We conclude that the smaller mean number of nuclei in the triploid eggs is a genuine phenomenon, not due solely to sampling error.

The best estimate of the value of the $3n/2n$ ratio, as shown in the detailed analysis on p. 547, is 0.78, and lying between 0.72 and 0.85, with a one in twenty probability of these not being the outside limits, or between 0.69 and 0.87, with a one in a hundred probability of error. The same analysis shows also that there is no evidence that the different ratios encountered from mouse to mouse are attributable to any factor other than sampling error.

(c) Tetraploid eggs

The data for tetraploid eggs may be examined in the same way as for triploids. In Table 1 there are six mice containing both diploid and tetraploid eggs. The six $4n/2n$ ratios are all below a value of 1.0, their average being 0.55. The ratio of the total of the six tetraploid means to the total of the six diploid means is 0.58. As shown on p. 547, the relatively smaller mean number of nuclei in tetraploids as compared with diploids seems to represent a genuine difference, with a chance of less than one in a thousand of being due solely to sampling error. The best estimate of the $4n/2n$ ratio is 0.52, lying between 0.41 and 0.67, with a chance of one in twenty of these not being the outside limits; a more stringent estimate of the limits is 0.38 and 0.73, with a one in a hundred chance of error. The detailed analysis also shows that there is no evidence that the differences in the ratios from mouse to mouse are due to any factor other than sampling error.

(d) Hexaploid eggs

As shown in Table 1, the two mice SiL/75 and V/94.3 each contained one hexaploid egg (nuclear numbers respectively 4 and 12), as well as several diploid eggs. The hexaploid with 4 nuclei contained fewer nuclei than the 6 diploid eggs in the same mouse, and was the least developed egg of all the 222 $3\frac{1}{2}$ -day diploid, triploid and tetraploid eggs described in this paper. The hexaploid with 12 nuclei contained fewer nuclei than the 3 diploid eggs in the same mouse; it also contained fewer nuclei than any of the 165 diploid eggs, fewer (with one tie) than in the 49 triploid eggs, and fewer (with one tie and one exception) than in the 8 tetraploids. It seems reasonable to conclude that hexaploid eggs in general contain fewer nuclei than diploids, triploids and (probably) tetraploids of the same age, and that exceptions are due to sampling error. The small number of observations, the low absolute value of the cell number in the hexaploids, and an evident disparity in the $6n/2n$ ratios of the two mice, seem to render an elaborate mathematical analysis undesirable for the present. The geometric mean $6n/2n$ ratio (0.19), with no standard error attached, will be taken as our best estimate of the true $6n/2n$ ratio.

(e) Detailed analyses of the sources of variation in the number of nuclei of triploid and tetraploid eggs

These analyses were performed for two reasons: (a) to obtain a clearer picture of the various sources of variation in nuclear number and, in particular, to assess the not easily visualized interaction term, and (b) to provide, with fiducial limits of probability, a best estimate of the $3n/2n$ and $4n/2n$ ratios.

Nuclear number in the developing mouse egg does not increase steadily, but tends to remain at 8, 16, 32 and so on, between successive cleavage waves. As a result, the variance would be expected to rise in proportion to the absolute magnitude of the observations. A logarithmic transformation was therefore carried out with the intention of equalizing the intervals between 8, 16, 32 and so on, and of rendering

variance independent of absolute magnitude. A further advantage of the transformation is that the mean difference between logarithmic mean nuclear number in diploid and polyploid eggs, together with the standard error of the mean difference, can very simply be transformed back into a *ratio* of the means, together with a fiducial limit of probability. In addition, the transformation lessens the undue weight which the few $4\frac{1}{2}$ - and $5\frac{1}{2}$ -day eggs, with their numerous nuclei, would otherwise exert on means.

Table 3. *Analyses of variance of the number (transformed into natural logarithms) of nuclei of diploid and polyploid eggs*

Source of variation	Triploid/diploid comparison		Tetraploid/diploid comparison	
	D.F.	Mean square	D.F.	Mean square
Between mice	46	0.6653*	5	0.8770*
Between diploid and polyploid eggs	1	2.0686*	1	2.1362*
Interaction	46	0.0922†	5	0.1557†
Between eggs of the same type of ploidy, within mice (error term)	109	0.0648	18	0.0718

* Significant at the 0.1 % *P* level in comparison with the error term.

† Not significant at the 5 % *P* level

Since disproportionate numbers were present in the subclasses of the analyses of variance, and it was not evident from inspection whether interaction was present, the method of weighted squares of means was used, assuming interaction (Snedecor, 1948, p. 299).

In the triploidy analysis (Table 3), data were taken from forty-seven mice, which contained altogether 148 diploid and 55 triploid eggs. Differences between mice (46 degrees of freedom) were highly significant at the 0.1 % *P* level. This presumably reflects, among other possible factors, that the times of copulation and fertilization were known only approximately, and that there was a consequent variation between mice in the general level of development of the eggs. The difference between the diploid and triploid means (D.F. 1) was highly significant at the 0.1 % *P* level; this confirms the result of the simpler analysis (p. 544) in which it was shown that the distribution of the 47 $3n/2n$ ratios differed significantly from a chance distribution around unity. The interaction term (D.F. 46) was not significant; hence there is no evidence that the differences between the 47 ratios are due to anything other than sampling error. The constitution of the error term is threefold: (a) a large and probably irreducible natural variation in nuclear number between eggs of the same chromosome number in the same mouse, quite evident in the fresh material; (b) an unknown variation due to accidental loss of nuclei during preparation; and (c) error in the counting of nuclei, believed to be negligible, for damaged or weakly stained eggs were excluded from the data. None of these three elements of the error term seems likely to affect diploid eggs more than triploids, or triploids more than diploids, and it is concluded that the error term is a valid and, in fact, necessary basis, on which to judge the significance of the main factors and their interaction. In any case,

the differences between mice, or between types of ploidy, remain significant at the 0.1% P level even when the interaction itself is used as error term.

In obtaining our best estimate of the true $3n/2n$ ratio, the mean logarithmic $2n-3n$ difference in each mouse was weighted by $(n_1+n_2)/n_1n_2$, where n_1 and n_2 are the numbers of diploid and triploid eggs respectively. The weighted mean so obtained was 0.2502. Its standard error, for 109 D.F., was 0.0428 (obtained by dividing the error mean square by 35.371, the latter being the sum of the weighting coefficients). Taking anti-logarithms, the mean $3n/2n$ ratio is found to be 0.78, with fiducial limits of 0.72-0.85 (to two places of decimals) at the 5% P level, or 0.69-0.87 at the 1% P level.

A similar analysis was performed with the six mice that contained diploid and tetraploid eggs, and similar results obtained (Table 3). Differences between mice, and between types of ploidy, were highly significant at the 0.1% P level, in comparison with the error term, and significant at the 5% P level in comparison with the interaction. The interaction was not significant in comparison with the error term. The best estimate of the mean logarithmic $2n-4n$ difference, for 18 D.F., was 0.6495 ± 0.1150 . Taking anti-logarithms, this means that the best estimate of the $4n/2n$ ratio is 0.522, with fiducial limits of 0.41-0.67 (to two places of decimals) at the 5% P level, or 0.38-0.73 at the 1% P level.

The mean squares in the tetraploidy analysis are slightly higher than in the triploidy analysis, as might be expected from the smaller number of degrees of freedom available in the tetraploidy analysis. Apart from this, the mean squares in the two analyses are of the same order of magnitude for any particular source of variation.

(f) Combination of results

In Fig. 1, the best estimates of the $1n/2n$, $3n/2n$, $4n/2n$ and $6n/2n$ ratios (as derived in the previous sections) are plotted against the number of chromosome sets. There is evidently a decrease in the ratio with increased number of chromosome sets. We have seen that the mean $3n/2n$ and $4n/2n$ ratios differ significantly from unity. They also differ significantly from one another, the mean difference in logarithmic units being 0.399 ± 0.123 , and therefore highly significant ($P=0.01-0.001$). (It should be noted that the diploid eggs of one mouse—serial number 269—were used in obtaining both the $3n$ and $4n$ estimates; this means, however, only that the true level of significance of the $3n/2n-4n/2n$ difference is likely to be even greater than calculated.) The plots for the $1n/2n$ and $6n/2n$ ratios, based on few observations, are necessarily approximate, but follow the same trend as those for the better known $3n/2n$ and $4n/2n$ ratios. We conclude that *the mean number of nuclei in polyploid eggs of the same age is approximately in inverse proportion to the number of chromosome sets present*. This may be appreciated visually in Fig. 1, where the curve derived from observations is seen to be rather close to the theoretical curve expressing an exact inverse proportion. Interpolation between the $1n/2n$ and $3n/2n$ plots from observations gives a value very close to 1.0, which must necessarily express the ' $2n/2n$ ' ratio.

The word 'approximately' was used in the above conclusion for two reasons: (a) only three of the fifty-six mice contributed to estimates of the $1n/2n$ and $6n/2n$

ratios, the results being principally those obtained from the $3n/2n$ and $4n/2n$ ratios; and (b) the $3n/2n$ ratio (0.78), although of the same order as the theoretical value of

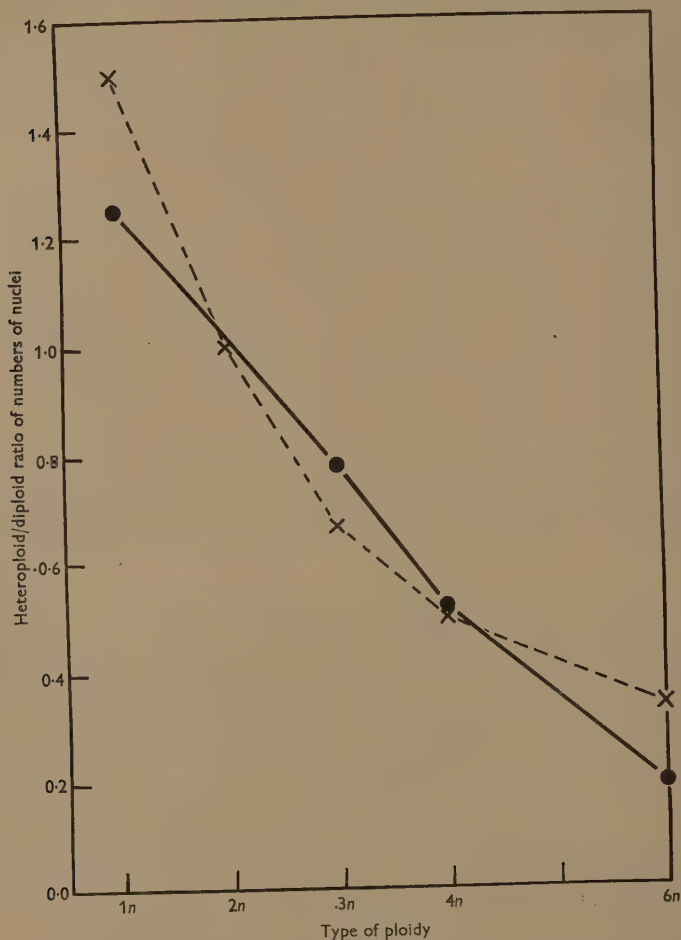


Fig. 1. Relation of the number of chromosome sets to the heteroploid/diploid ratio of cell number. Black dots, joined by full lines, are best estimates from the data. Crosses, joined by broken lines, are theoretical ratios on the assumption that the mean number of cells in heteroploids, relative to the number in diploids, is inversely in proportion to the number of chromosome sets. The trend of the theoretical curve is in good agreement with the experimental curve.

0.67, nevertheless differs significantly from it; the $4n/2n$ ratio (0.52), however, does not differ significantly from a theoretical 0.50 ratio.

IV. DISCUSSION

The results have been expressed so far in terms of the objects actually observed, i.e. nuclei. It is known that polynucleate cells are rare or unknown at the stages of development examined. The number of nuclei counted in a preparation will therefore be equated in this discussion with the number of cells present.

The conclusion that the mean number of cells in polyploid mouse eggs, relative to diploids of the same age, is approximately in inverse proportion to the number of chromosome sets present, parallels closely the situation in amphibian larvae (Fankhauser, 1945; Fischberg, 1944, 1948; Briggs, 1947). In mice the $3n/2n$ ratio is rather greater than the theoretical expectation (0.78 instead of 0.67) at the early stages examined by us. Only one haploid mouse egg was found, and, in view of the great variation found between mouse eggs even of the same type of ploidy and in the same mouse, we cannot state whether haploid mouse eggs in general follow an inverse proportion law; for triploid, tetraploid and hexaploid eggs, however, the inverse relation seems quite clear.

The polyploid eggs were of normal cytological appearance, and we have observed in other work (Fischberg & Beatty, 1951*b*) that triploid mouse eggs can survive to at least $9\frac{1}{2}$ days after fertilization. It seems unlikely therefore that the lesser number of cells in polyploid eggs can be due to the eggs being moribund or dead.

In the detailed analyses of the $3n/2n$ and $4n/2n$ ratios, there was no evidence that the different ratios encountered from mouse to mouse were due to anything other than sampling error. Nor was any correlation evident when the $3n/2n$ or $4n/2n$ ratios of each mouse were plotted against the average cell number of each mouse (the latter figure being the average of the mean of the diploid and the mean of the triploid or tetraploid eggs in the same mouse). A reasonable number of mice were involved (52), and we may form the provisional conclusion that both the $3n/2n$ and $4n/2n$ ratios remain fairly constant over the ranges observed (early morula to late blastula). This suggests that the lesser number of cells in triploid and tetraploid eggs, relative to diploid eggs, arises at an early cleavage stage. In Amphibia, the first cleavage mitosis occurs at the same time in diploid and triploid eggs of *Triturus viridescens* (Fankhauser & Godwin, 1948). In triploid *Rana pipiens* (Briggs, 1947), the first three cleavages take place at the same times in diploids as in triploids. Our results with mice do not show the time of origin of the difference, but do indicate that it arises early. The difference in cell number between diploids and tetraploids is quite evident in Amphibia at an early larval stage (Fankhauser, 1945), but its exact time of origin is not clear. Most of the tetraploid mouse eggs quoted in the present paper were produced by a hot shock intended to suppress the first cleavage division; thus, at least at this early stage of development, tetraploid mouse eggs would be expected to contain half the number of cells of diploids.

We have seen evidence that the lesser number of cells in triploid mouse embryos arises in early cleavage, and in tetraploids at the first cleavage. We may now consider the interrelationships of cell size, cell number and body size in later embryos. We have observed (Fischberg & Beatty, 1951*b*) that triploid $9\frac{1}{2}$ -day mouse embryos are

smaller than diploids in the same mouse. They must be assumed therefore to contain fewer cells than diploids, and particularly so since cells in polyploid organisms of recent origin are larger than in diploids. (In races with increased chromosome number which have passed through many generations, cell size may regulate in time towards the normal, as in mosses (von Wettstein, 1937).) We feel therefore that triploidy in the $9\frac{1}{2}$ -day mouse embryo (and perhaps at least up to birth, unless new factors intervene) follows the pattern of the triploid amphibian larva and adult, in which the triploid contains fewer and larger cells, but body size is not greater than in diploids. This contrasts with the pattern of some plants and invertebrates, where the polyploid cells are also larger, but the number of cells in polyploids and diploids is about equal, and the organism is of giant size. The adult triploid rabbits reported by Häggqvist & Bane (1950*a*), however (see also Häggqvist & Bane, 1950*b*, *c*, and Melander, 1950; some criticisms have been raised of the interpretation of data made by these authors—see Beatty & Fischberg (1950) and Nachtsheim (1950*a*, *b*)) seem, unexpectedly, to follow the plant rather than the amphibian pattern.

In plants and invertebrates, triploids of recent origin are usually larger than diploids; in Amphibia they are of the same size; in our $9\frac{1}{2}$ -day mouse embryos they are smaller. Tetraploid plants and invertebrates are usually larger than diploids and triploids; in Amphibia, tetraploids start the same size, are retarded in mid-larval development (possibly for reasons of physiological inefficiency), and do not reach the same size as diploids or triploids. These facts suggest that the size of polyploid organisms, relative to diploids, decreases the higher they are in the systematic scale. Post-implantation tetraploid mammals are unknown at the time of writing; if they are found, we feel that they are likely to be smaller than diploids.

A possible intra-uterine competition between polyploids and diploids has been envisaged (Beatty & Fischberg, 1951*b*; Fischberg & Beatty, 1951*a*, *b*), whereby the implanted polyploids, with their smaller number of cells, would be at a disadvantage in comparison with diploids. However, in nine of the forty-seven mice with triploid eggs shown in Table 1, the mean number of cells in the diploids was less than or equal to the mean number of cells in the triploids. It seems therefore that a certain number of mice exist in which polyploid eggs, by chance, do not have a lesser number of cells than diploids, and these polyploids might therefore be exempt from intra-uterine competition.

We may now examine the use of nuclear counts as a possible aid in identifying polyploid eggs. If we select one egg at random from each mouse in Table 1, we shall obtain fifty-nine eggs among which will be about a quarter of the total polyploid eggs. If, however, we select from each mouse the egg with the smallest number of nuclei, we shall have included about half of the total number of polyploid eggs. The essential method of determining polyploidy lies in the counting of chromosomes, but the counting of nuclei has evidently a definite though limited use as preliminary or confirmatory evidence.

V. SUMMARY

1. Cell number, determined by counting the nuclei, has been studied in pre-implantation haploid, diploid, triploid, tetraploid and hexaploid eggs of *Mus musculus*, recovered usually $3\frac{1}{2}$ days after fertilization. Most of the data concern triploid and tetraploid eggs, from the early morula to late blastula stages.

2. The mean number of cells in polyploid eggs (and perhaps in haploids), relative to the number in diploids of the same age, is approximately in inverse proportion to the number of chromosome sets present. Thus, the ratio of cell number in polyploid eggs to cell number in diploid eggs decreases with an increase in the number of chromosome sets.

3. There was no evidence for any one type of heteroploidy that the differences in ratios encountered from mouse to mouse were due to anything other than sampling error, i.e. the ratios remained fairly constant from the early morula to the late blastula. This suggests that the lesser number of cells in polyploids is already apparent in early cleavage.

4. These findings are compared with the parallel situation in Amphibia.

5. The counting of nuclei can play a role, but only a limited one, in the identification of polyploid eggs.

We wish to thank Prof. C. H. Waddington, F.R.S., for helpful interest in the work. We are indebted to Mr R. M. Mabon for technical assistance. We are particularly indebted to Mr A. Robertson for advice on statistical procedure, and to Mr W. Russell for carrying out most of the computations. One of us (M. F.) acknowledges grants from the Animal Breeding and Genetics Research Organization and the Schweizerische Stiftung für biologisch-medizinische Stipendien, and wishes to thank the former Organization for its hospitality.

REFERENCES

- BEATTY, R. A. & FISCHBERG, M. (1949). Spontaneous and induced triploidy in pre-implantation mouse eggs. *Nature, Lond.*, **163**, 807-8.
- BEATTY, R. A. & FISCHBERG, M. (1950). 'Polyploidy in rabbits.' *Nature, Lond.*, **166**, 238-9.
- BEATTY, R. A. & FISCHBERG, M. (1951*a*). Heteroploidy in mammals. I. Spontaneous heteroploidy in pre-implantation mouse eggs. *J. Genet.* **50**, 345-59.
- BEATTY, R. A. & FISCHBERG, M. (1951*b*). Heteroploidy in mammals. III. Induction of tetraploidy in pre-implantation mouse eggs. *J. Genet.* (in the Press).
- BRIGGS, R. (1947). The experimental production and development of triploid frog embryos. *J. Exp. Zool.* **106**, 237-66.
- FANKHAUSER, G. (1945). The effects of changes in chromosome number on amphibian development. *Quart. Rev. Biol.* **20**, 20-78.
- FANKHAUSER, G. & GODWIN, D. (1948). The cytological mechanism of the triploidy-inducing effect of heat on eggs of the newt *Triturus viridescens*. *Proc. Nat. Acad. Sci., Wash.*, **34**, 544-51.
- FISCHBERG, M. (1944). Veränderungen der Chromosomenzahl bei *Triton alpestris* nach Kältebehandlung der Eier. *Rev. suisse Zool.* **51**, 430-6.
- FISCHBERG, M. (1948). Experimentelle Auslösung von Heteroploidie durch Kältebehandlung der Eier von *Triton alpestris* aus verschiedenen Populationen. *Genetica*, **24**, 213-329.
- FISCHBERG, M. & BEATTY, R. A. (1950*a*). Anfänge einer genetischen Analyse der spontanen Heteroploidie bei Mäusen. *Arch. Klaus-Stift. VererbForsch.* **25** (1/2), 22-7.
- FISCHBERG, M. & BEATTY, R. A. (1950*b*). Experimentelle Herstellung von polyploiden Mausblastulae. *Arch. Klaus-Stift. VererbForsch.* **25** (1/2), 54-5.

- FISCHBERG, M. & BEATTY, R. A. (1951*a*). Heteroploidy in mammals. II. Induction of triploidy in pre-implantation mouse eggs. *J. Genet.* (in the Press).
- FISCHBERG, M. & BEATTY, R. A. (1951*b*). Spontaneous heteroploidy in mouse embryos up to mid-term. (In the Press.)
- HÄGGQVIST, G. & BANE, A. (1950*a*). Polyploidy in rabbits induced by colchicine. *Nature, Lond.*, **165**, 841-3.
- HÄGGQVIST, G. & BANE, A. (1950*b*). Studies in triploid rabbits produced by colchicine. *Hereditas, Lund*, **36**, 329-34.
- HÄGGQVIST, G. & BANE, A. (1950*c*). Chemical induction of polyploid breeds of mammals. *K. svenska VetenskAkad. Handl.* **1**, 1-12.
- MELANDER, Y. (1950). Chromosome behaviour of a triploid adult rabbit, as produced by Häggqvist and Bane after colchicine treatment. *Hereditas, Lund*, **36**, 335-41.
- NACHTSHEIM, H. (1950*a, b*). Riesenkaninchen mit erhöhter Chromosomenzahl? *Dtsch. Kleintier Zucht.* (a) no. 23, p. 2; (b) no. 24, p. 6.
- SNEDECOR, G. W. (1948). *Statistical Methods*, 4th ed. Iowa.
- VON WETTSTEIN, F. (1937). Experimentelle Untersuchungen zum Artbildungsproblem. I. Zellgrößenregulation und Fertilwerden einer polyploiden Bryum-Sippe. *Z. indukt. Abstamm.-u. VererbLehre*, **74**, 34-53.

THE SWIMBLADDER AND THE VERTICAL MOVEMENTS OF TELEOSTEAN FISHES

I. PHYSICAL FACTORS

By F. R. HARDEN JONES

Department of Zoology, Queen Mary College, University of London

(Received 20 April 1951)

(With Three Text-figures)

CONTENTS

	PAGE
Introduction	553
The relative volume of the swimbladder	554
The change in density.	555
The restriction to rapid vertical movements	555
The restriction to slow vertical movements	556
The experimental work	557
Physical factors	558
The resistance offered to the expansion of the swimbladder gas	558
The percentage volume of the swimbladder	560
Density determinations	561
Rupture of the bladder wall	562
Discussion	563
Summary	564
References	565

INTRODUCTION

Moreau (1876) was one of the first to suggest that the vertical movements of a teleost are restricted by the presence of a swimbladder which increases or decreases in volume when the fish moves up or down in the water. The reason for this is that when the fish passes from one level to another it is subjected to a change of hydrostatic pressure. If the fish moves above the level at which it is in hydrostatic equilibrium with its environment, the decrease in hydrostatic pressure (1 atm. for about every 10 m. of water) leads to an expansion of the swimbladder. As a result the fish as a whole becomes larger and therefore less dense than the water and tends to rise to the surface. Conversely, if it swims into deeper water the bladder decreases in volume, with the result that the fish displaces less water and tends to sink. A large vertical movement above or below the plane of equilibrium might result in the fish being carried up to the surface or down to the bottom. Too great a pressure reduction might lead to the rupture of the bladder wall. This danger would appear to be a real one; fish taken by line or trawl often arrive at the surface visibly distended and they are described as 'blown'. If they are taken from deep water the enlarge-

ment of the bladder may lead to the eversion of the stomach through the mouth (Hickling, 1927) or to a rupture of the bladder wall (Graham, 1924). Moreau believed that this danger would be less for a physostome than for a physoclist, as while the former could get rid of the excess gas rapidly by using the pneumatic duct as an escape valve, the latter could do so by the slower process of gas resorption which may take several hours. He concluded that a swimbladder, and in particular a closed one, would restrict the vertical movements of a teleost by confining it at any given moment to a definite bathymetrical range.

There is very little information available from which any conclusions can be drawn about the extent to which the presence of a swimbladder restricts the vertical movements of a teleost. The problem is of interest as it is known that various teleosts undertake diurnal, seasonal or other periodic migrations involving vertical displacements (Murray & Hjort, 1912; Vedel Tåning, 1918; Pearse & Achtenberg, 1918; Hickling, 1925; Johansen, 1925; Russell, 1928; Southern & Gardiner, 1932; Allen, 1935; Hasler & Bardach, 1949). Does the swimbladder restrict the magnitude or speed of these movements? Furthermore, it has been suggested that fish may be the scatterers responsible for the diurnal migration shown by the deep scattering layer (Chapman, 1947; Dietz, 1948; Hersey & Moore, 1948; Johnson, 1948; Moore, 1950; Boden, 1950). If an estimate could be made of the restriction that the swimbladder imposes on vertical movements the information might be of interest in connexion with this and other scattering layers.

In the present paper an attempt is made to determine the extent to which the presence of the swimbladder restricts the vertical movement of the perch, *Perca fluviatilis* Lin. Before passing on to the experimental work the problem is first considered from a theoretical point of view, and it is shown how the swimbladder might restrict the vertical movements of a teleost and on what factors the extent of the restriction will depend.

The relative volume of the swimbladder

The average density of a fish, excluding the swimbladder, may be taken as 1.076 (Taylor, 1921). A fresh-water physoclistous fish in hydrostatic equilibrium with water of density 1.000 and occupying a total volume of 100 ml. can be shown to have a swimbladder of approximately 7 ml. For if x is the volume of the swimbladder then

$$\begin{aligned} 1.076 (100 - x) &= 100, \\ x &= 7.06, \text{ say } 7 \text{ ml.} \end{aligned}$$

Similarly, if the fish were in hydrostatic equilibrium with sea water of density 1.026, it could be shown that the swimbladder would occupy a volume of about 5 ml. So when the swimbladder functions as a hydrostatic organ it should occupy about 7% of the total volume of a fresh-water teleost, but slightly less, about 5%, of that of a marine teleost. It is interesting to note that the figures given by Plattner (1941) for the relative size of the swimbladder in various fresh-water and marine teleosts (viz. *Tinca vulgaris* 7.7%, *Carassius auratus* 8.3%, *Zeus faber* 5.6%, *Gadus luscus* 4.9%) compare well with those calculated theoretically.

The change in density

A fresh-water fish in hydrostatic equilibrium at a depth of 20 m. is under a total pressure of 3 atm.; the 20 m. level may be called the fish's plane of equilibrium. According to Moreau, when the fish swims up, the swimbladder and thus the volume of the fish as a whole increases, with the result that its density decreases. Whether or not the gas obeys Boyle's law will depend on the passive resistance that the bladder wall and surrounding tissues offer to its expansion and on the degree to which the fish can actively control the volume of its swimbladder by muscles lying in or about the bladder wall. If resistance is offered to the expansion of the swimbladder gas, the density change will be less than that which would occur if the gas were to expand unhindered.

The restriction to rapid vertical movements

Confining the argument to movements which the fish might make above its plane of equilibrium, a distinction can be made between *rapid* movements characteristic of normal locomotion, and *slow* movements which occur during the course of diurnal, seasonal or other periodic migrations. By a rapid movement is meant one during which the fish moves so quickly from one level to another that it will be unable to make any appreciable adjustment in the volume of its swimbladder.

If a fish moves rapidly above its plane of equilibrium for a small distance, its volume increases and its density decreases. To maintain its station at the new and higher level the fish must resist its buoyancy by the action of its fins until its density has been restored to that of the environment by the removal of the excess gas from the swimbladder. But the force that the fish can resist by the compensatory movements of its fins is limited. So that the fish would be carried to the surface if it made a rapid movement that produced, through the decrease in density, a force greater than that which could be resisted by the fins. Furthermore, a large pressure reduction might lead to a rupture of the bladder wall. The limit to a rapid ascent will therefore depend on the force that can be resisted by the compensatory swimming movements. The pressure reduction which produces the critical force will depend on the change in the density of the fish. This in turn will depend on the resistance offered to the expansion of the bladder gas, and the amount by which the gas expands will also depend on the original volume of the swimbladder. And other things being equal, as the swimbladder is relatively greater in fresh-water than in marine teleosts, its presence will impose a greater restriction on the movements of the former than on those of the latter.

One further point may be mentioned. If the fish swam up to the full limit of its range, it does not seem likely that it would remain there for long. Having to compensate vigorously to maintain its station, it would soon become fatigued. A rapid movement would appear to lead to no more than an excursion from the plane of equilibrium such as might occur in the pursuit of prey or the avoidance of predators. But this would not prevent the fish from making smaller movements which involve

density decreases for which it could compensate satisfactorily. It could then remain at the new level. There would then be a narrow zone within which the fish could swim freely and a wider zone into which it would make only temporary excursions.

The restriction to slow vertical movements

If the fish were to migrate from deep to shallow water it must move fairly slowly from one level to another. Suppose the fish is in hydrostatic equilibrium at a depth of y m. below the surface. The pressure P in atmospheres at that depth will be given by the equation

$$P = ky + 1, \quad (1)$$

where k is a constant for the increase in pressure with depth. If the fish moves up through the small distance $-\delta y$, then the pressure change δP will be

$$\delta P = k\delta y, \quad (2)$$

and in the limit

$$\frac{dP}{P} = \frac{k dy}{ky + 1}. \quad (3)$$

If the fish is to remain in hydrostatic equilibrium as it moves up, the rate of gas resorption must keep pace with that of the vertical movement. The time dT that it will take the fish to pass through the distance $-\delta y$ will therefore depend on B , the rate at which it can adjust its density when subjected to the relative pressure reduction $-dP/P$,

$$dT = -B \frac{dP}{P} \quad (4)$$

$$= -B \frac{k dy}{ky + 1}. \quad (5)$$

If the fish is considered as moving from a low to a higher level in a series of little steps, then the total time T taken to pass from a depth l_1 of pressure P_1 to a shallower depth l_2 of pressure P_2 is given by the equation

$$T = - \int_{l_1}^{l_2} B \frac{k dy}{ky + 1}. \quad (6)$$

Integrating between the limits l_1 and l_2

$$T = -B \log_e \frac{kl_2 + 1}{kl_1 + 1} \quad (7)$$

$$= -B \log_e \frac{P_2}{P_1} \quad (8)$$

$$= B \log_e \frac{P_1}{P_2}. \quad (9)$$

It has been assumed that the fish is always in hydrostatic equilibrium as it moves up and does not have to maintain its station with compensatory movements. But this is not the quickest way in which it could move from deep to shallow water. Suppose that the fish moved rapidly above its plane of equilibrium for such a distance that the pressure reduction led to a decrease in density for which it could compensate

satisfactorily. If the fish were migrating from deep to shallow water the fastest way in which it could complete the movement would be to swim up for this distance rapidly and then to proceed slowly, adapting the volume of its swimbladder to avoid any further decrease in density. For example, suppose the fish could maintain its station when the total pressure to which it was adapted was reduced by one-tenth. If it swims up for this distance rapidly, it is no longer adapting itself to the pressure difference between P_1 and P_2 but to the smaller difference between $\frac{9}{10}P_1$ and P_2 . Substituting for P_1 in equation (9),

$$T = B \log_e \frac{\frac{9}{10}P_1}{P_2} \quad (10)$$

$$= B \log_e \frac{P_1}{P_2} - \log_e \frac{10}{9}, \quad (11)$$

or in a general form
$$T = B \log_e \frac{P_1}{P_2} - C, \quad (12)$$

where B and C are constants for a particular fish, B being a factor for the rate of adaptation to a reduction in pressure and C being a correction for the initial rapid movement of the fish.

So for a physoclist there are two restrictions which the presence of the swimbladder might impose to vertical movements involving a reduction in pressure. First, a restriction on the extent of a rapid movement, and secondly, a restriction on the speed with which the fish can migrate from one level to another. In a similar way it can be shown that the swimbladder restricts movements involving an increase in pressure. So the fish may be considered as having a definite bathymetrical range within which it can pass rapidly from one level to another. But if it were to move outside its range, the speed with which it could swim up or down would depend on the rate at which it could adjust the volume of its swimbladder when subjected to changes in hydrostatic pressure. On the other hand, a physostome would be able to move freely above its plane of equilibrium, but its descents would be restricted in the same way as those of a physoclist.

The experimental work

The restriction that the swimbladder imposes to vertical movements involving a reduction in pressure will depend on physical factors such as

- (1) The resistance that the bladder and body wall offer to the expansion of the bladder gas.
- (2) The percentage volume of the swimbladder and the density change of the fish when it is subjected to a reduction in pressure.
- (3) The pressure reduction that leads to the rupture of the bladder wall.

In rapid movements the compensatory ability of the fish must be considered, and in slow movements the speed with which the fish can accommodate itself to pressure changes. To study the physical factors experiments were made on three physoclists: the perch and two marine fish, the wrasse, *Crenilabrus melops*, and the five-bearded rockling, *Onos mustela*. Control experiments were made with the dragonet

Callionymus lyra, which has no swimbladder. Experiments on the restriction to rapid and slow movements were confined to perch. These experiments could only be made with reductions in pressure. When subjected to an increase in pressure the perch would rest quietly on the bottom of the tank and no observations could be made on their behaviour or rate of gas secretion. But with decreases in pressure everything was quite satisfactory and results were obtained.

The experimental work is presented in three sections:

- (1) Physical factors.
- (2) The restriction to rapid movements.
- (3) The restriction to slow movements.

Section 1 is included here, while the other two appear in a following paper.

PHYSICAL FACTORS

As Jacobs (1933) has shown that the perch is unable to control the volume of its swimbladder by muscular action, experiments were made to determine what passive resistance might be offered to the bladder gas in this fish. The percentage volume of the swimbladder was found in the perch, wrasse and rockling and the results correlated with the density changes shown by these fish when they were subjected to reductions in pressure. Experiments were made with perch to determine the pressure reduction that led to the rupture of the bladder wall.

The density measurements of the perch were made in London with fish obtained from dealers. Other experiments with this species were made at Wray Castle on perch trapped in Windermere. The work with the marine species was done at the Plymouth Laboratory. All the fish used were about 10 cm. long.

The resistance offered to the expansion of the swimbladder gas

Method

A perch was subjected to a pressure reduction and the volume change measured. The fish was then killed and the volume of the swimbladder determined. By expressing the observed volume changes as percentages of the original volume of the swimbladder, the experimental results may be compared directly with those that would be obtained with a gas expanding freely under the same conditions. If any resistance was offered to the expansion of the bladder gas, the experimental results would be less than those calculated from Boyle's law.

The volume change of the perch. The volume change was measured by a displacement method. The fish was put into a flat-bottomed glass tube filled with water and fitted with a narrow tube graduated in 0.1 ml. The volume change of the fish was measured by observing the rise in the level of the water meniscus. To avoid errors due to distorsion, the apparatus was placed in a Kilner jar connected to a manometer and vacuum pump as shown in Fig. 1. The pressure was reduced by 30 cm. Hg in about 2-3 min., readings of the meniscus level being taken after every 5 cm. reduction. The rate at which the fish can remove gas from the swimbladder is so slow as to have no effect on the results of these experiments.

The volume of the swimbladder. The volume of the swimbladder could not be found directly by displacement as it was too delicate to remove easily. So the fish was killed, gutted and the head and tail removed leaving the bladder attached to the trunk of the fish. The trunk and bladder were put into a density bottle improvised from a 100 ml. weighing bottle. The bottle, water, trunk and bladder were weighed to the nearest 0.1 g. The trunk was then removed, the bladder torn away and a second weighing made with the trunk in the bottle. The difference between the two weighings gave the weight of water displaced by the swimbladder.

The passive resistance offered to the expansion of the bladder gas was calculated from the results of experiments made on ten perch.

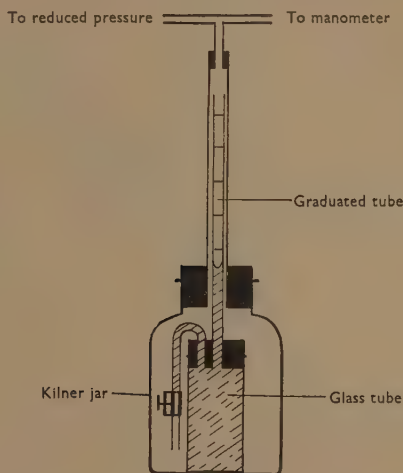


Fig. 1. Apparatus to measure the change in volume of a fish subjected to a reduction in pressure.

Table 1. *Volume changes of perch when subjected to different pressure reductions expressed as a percentage of the original volume of the swimbladder*

Pressure reduction in cm. Hg	...	5	10	15	20	25	30
Volume change expected (%)		7	15	25	36	49	65
Volume change found (%), mean of ten experiments		7	15	23	31	42	52

Results

The results are set out in Table 1, where the observed volume changes, expressed as percentages of the original volume of the swimbladder, are compared with those to be expected if the gas followed Boyle's law, and shown graphically in Fig. 2. The resistance offered to the expansion of the bladder gas by the bladder wall and surrounding tissues is only apparent when the pressure is reduced by more than 10 cm. Hg.

*The percentage volume of the swimbladder**Method*

The percentage volume of the swimbladder was found after determining the volume of the fish and that of its swimbladder. Experiments were made with perch, wrasse and rockling.

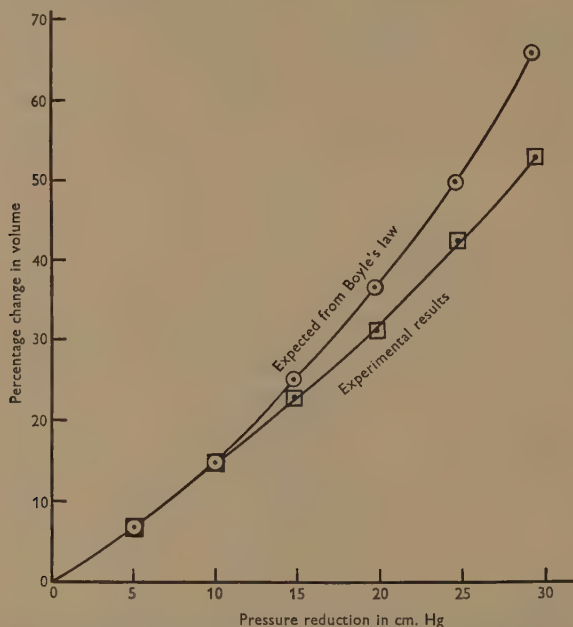


Fig. 2. Percentage change in volume of the perch's swimbladder at different pressure reductions. Expected and experimental results. Data from Table 1.

The volume of the fish. This was found by displacement. After a standard drying procedure a fish was dropped into a measuring cylinder holding a known amount of water. The cylinder was then filled up to the top mark. Knowing the amount of water added to fill the cylinder and that already in it, the volume of the fish was found by subtracting the sum of the two volumes from the capacity of the cylinder. This roundabout method was better than dropping the fish into the cylinder and noting the change in meniscus level directly. If there is sufficient water to cover the fish some is always lost by the splashing of the tail as it enters the water.

The volume of the swimbladder. The volume of the perch's swimbladder was found in the manner described earlier. In the case of the wrasse and the rockling it was found by collecting and measuring the volume of the gas in the bladder.

The percentage volume of the swimbladder was found in twenty-nine male perch, twenty wrasse and sixteen rockling.

Results

The results are set out in Table 2 below.

Table 2. *The percentage volume of the swimbladder*

	<i>Perca fluviatilis</i>	<i>Crenilabrus melops</i>	<i>Onos mustela</i>
No. of determinations	29	20	16
Mean (%)	7.5	4.9	1.1
Standard deviation	0.9	0.7	0.8

Density determinations

Methods

Experiments were made with perch, wrasse, rockling and dragonet to determine the density changes undergone by these fish when they were subjected to pressure reductions. The density of a fish was found by determining its weight and volume at a known temperature. The fish was then subjected to a reduction in pressure and the increase in its volume measured. Its volume at a given pressure reduction was found by adding the observed volume change to the original volume of the fish. Assuming its weight to remain constant, the density of the fish at different pressures may then be calculated. Before a determination a fish was kept for 24 hr. in a tank filled with water to a depth of 60 cm. As this head of water exerts a pressure equivalent to about 4 cm. Hg on the bottom, the fish were considered as being adapted to a total pressure of $76 + 4 = 80$ cm. Hg.

Density determinations. Lowndes's (1937, 1941) displacement method was used for the wrasse, rockling and dragonet. A density bottle of known volume was weighed full of sea water, a fish inserted and the bottle weighed again. To find the volume of the fish the density bottle was filled with sea water and the contents washed into a 200 ml. volumetric flask which was made up to the mark with distilled water. The halide solution was titrated with silver nitrate. The density bottle was again filled with sea water and the fish inserted. The water and washings, made with isotonic sodium nitrate, were poured into a second 200 ml. flask. A second titration was made which is less than the first. The volume of the fish can be calculated from the results of the two titrations. Dr Lowndes very kindly loaned a calibrated density bottle and the use of a balance for these experiments.

While the principle of Lowndes's method is applicable to fresh-water animals (Lowndes, 1937) it was found more convenient to use a simpler method for the perch. It is less accurate than that devised by Lowndes but adequate for the purpose of the experiments. A density bottle was made from a 100 ml. weighing bottle, and its weight full of water was consistent to 0.01 g. The bottle was partly filled with water and weighed. After a standard drying procedure a perch was inserted and the bottle weighed again. The weight of the fish was found by subtraction. The density bottle was then filled with water and a third weighing made with the fish inside. The density of the fish could then be calculated. The weighings were made to the nearest 0.1 g.

The volume of the fish. After a density determination a fish was subjected to a reduction in pressure and the increase in its volume measured. The apparatus and method have already been described. The pressure was reduced by 30 cm. Hg, readings of the meniscus level being taken after every 5 or 10 cm. reduction.

From the results of the two series of experiments the density of a fish could be calculated at a given pressure reduction. Experiments were made with five fish of each species.

The sinking factor. The densities of fresh-water and marine animals may be compared by mean of the sinking factor (or s.f.), a term introduced by Lowndes (1937) which is numerically equal to 1000 times the quotient obtained by dividing the density of the animal by that of its environment. When a fish is in hydrostatic equilibrium the sinking factor will be 1000. From the experimental results the sinking factor of each fish was calculated, and for this purpose the density of Plymouth sea water was taken as 1.026 and that of fresh water as 1.000.

Results

The results are set out in Table 3, where the densities, sinking factors and changes in sinking factors are given for different pressure reductions. As might be expected, *Callionymus*, lacking a swimbladder, showed no change. The perch with the largest swimbladder showed the most change, while the rockling with the smallest swimbladder showed the least. This can be seen in Fig. 3, where the change in sinking factor is plotted against the pressure reduction.

Table 3. *Densities, sinking factors and change in sinking factors for perch, wrasse, rockling and dragonet at different pressure reductions*

Pressure reduction in cm. Hg ...		0	5	10	15	20	25	30
<i>Perca fluviatilis</i> , five experiments	Density	1.005	1.000	0.995	0.988	0.982	0.975	0.968
	Standard dev.	0.005	0.005	0.005	0.005	0.004	0.005	0.005
	Sinking factor	1005	1000	995	988	982	975	968
	Change in s.f.	0	5	10	17	23	30	37
<i>Crenilabrus melops</i> , five experiments	Density	1.032	1.029	1.026	1.021	1.017	1.012	1.007
	Standard dev.	0.002	0.002	0.003	0.002	0.003	0.003	0.004
	Sinking factor	1007	1003	1000	995	991	986	982
	Change in s.f.	0	4	7	12	16	21	25
<i>Onos mustela</i> , five experiments	Density	1.050		1.048		1.046		1.043
	Standard dev.	0.005		0.005		0.006		0.007
	Sinking factor	1024		1022		1020		1016
	Change in s.f.	0		2		4		8
<i>Callionymus lyra</i> , five experiments	Density	1.083			No change observed			
	Standard dev.	0.016			No change observed			
	Sinking factor	1056			No change observed			
	Change in s.f.							

Method

Rupture of the bladder wall

A perch was killed and the body cavity opened up. The fish was then subjected to a slow reduction in pressure until the bladder wall gave way. Experiments were made with twenty male and twelve female fish. These were kept in a shallow tank before use and were considered as being adapted to a pressure of 77 cm. Hg.

Results

From Table 4 it will be seen that the swimbladders of male perch ruptured when the pressure was reduced by 43 cm. Hg and those of females at a reduction of 45 cm. Hg. The difference has no statistical significance (t test, $0.2 > P > 0.1$), and pooling the data the mean pressure reduction comes to 44 cm. Hg. As the perch were adapted to a total pressure of 77 cm. Hg this reduction is approximately three-fifths of the total pressure to which they were adapted.

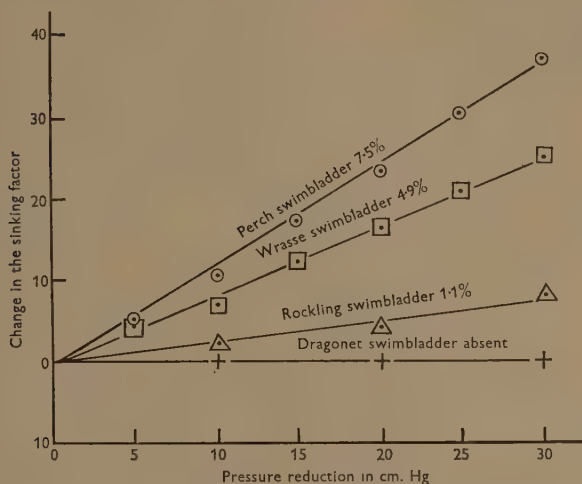


Fig. 3. Change in sinking factor for perch, wrasse, rockling and dragonet subjected to pressure reductions. Data from Table 3.

Table 4. Pressure reduction required to rupture the swimbladder wall of male and female perch adapted to a total pressure of 77 cm. Hg

Sex	No. of experiments	Pressure reduction in cm. Hg	Standard deviation
Males	20	43	4
Females	12	45	4

Discussion

When the swimbladder functions as a hydrostatic organ it should occupy a volume of about 7% in a fresh-water teleost and about 5% in a marine species. The correlation between a large swimbladder and a low sinking factor in the perch and the wrasse and a high sinking factor and a small swimbladder or its absence in the rockling and the dragonet was therefore to be expected. In the rockling the swimbladder would not appear to function as a hydrostatic organ, and both this fish and the dragonet swim with difficulty.

The changes in the sinking factors of the perch, wrasse, rockling and dragonet at pressure reductions of 10, 20 and 30 cm. Hg are in the average ratio of 7.5:5.2:1.5:0, while the relative sizes of their swimbladders are in the very similar ratio of 7.5:4.9:1.1:0. As can be seen in Fig. 3 there is a relation between the size of the bladder and the amount by which the sinking factor decreases when the fish are subjected to reductions in pressure. Other things being equal the larger the swimbladder, the greater the change in sinking factor. And as the swimbladder tends to be relatively larger in fresh-water than in marine teleosts, the experimental results show, as suggested earlier, that its presence might therefore restrict the movements of the former more than those of the latter. But any means by which the volume of the swimbladder might be reduced and the sinking factor still kept low, such as increasing the average fat content of the tissues (Taylor, 1921), would reduce its restrictive influence and thus be of advantage to a pelagic fish. A swimbladder with a thick wall capable of resisting the expansion of the bladder gas would have a similar effect. But the bladder wall of the perch is thin and offers little resistance to the expansion of the bladder gas, and the density of the fish changes considerably when it is subjected to a pressure reduction.

The swimbladder of the perch burst when the total pressure to which it is adapted is reduced by three-fifths. The results of these experiments have been confirmed by those of a series of trapping experiments made in Windermere (Jones, 1949), in which the bladders of over 600 perch were examined after the fish had been hauled rapidly to the surface from different depths. Fish brought to the surface from a depth of 13.7 m. (45 ft.) are subjected to a pressure reduction of three-fifths, and from the results of the laboratory experiments it would be expected that all the fish taken from a depth of 13.7 m. or more would have broken bladders, while those taken from shallower water would have their bladders intact. This was generally so. Now the rupture of the bladder wall is probably fatal, and about 90% of the fish taken from deep water soon die. It is therefore reasonable to suppose that in normal circumstances a perch would not make a rapid ascent that led to the rupture of its bladder wall.

To summarize the discussion: Experiments show that there is a relation between the relative size of the swimbladder and the change in the density of a fish when it is subjected to a reduction in pressure; that the bladder and body wall of the perch offer little resistance to the expansion of the bladder gas; and that the danger of the bladder wall rupturing might restrict the extent of a rapid movement.

SUMMARY

The vertical movements of a teleostean fish may be restricted by the presence of the swimbladder, which will increase or decrease in volume when the fish moves up or down in the water.

It is shown that the restriction that the swimbladder imposes to vertical movements involving a reduction in pressure will depend on physical factors such as

(1) The resistance that the bladder and body wall offer to the expansion of the bladder gas.

(2) The percentage volume of the swimbladder and the density change of the fish when it is subjected to a reduction in pressure.

(3) The pressure reduction that leads to the rupture of the bladder wall.

A distinction is made between rapid and slow movements. In the former the compensatory ability of the fish must be considered and in the latter the speed with which the fish can accommodate itself to pressure changes.

An equation is derived from which the minimum speed at which a physoclist can migrate from deep to shallow water can be calculated. To solve the equation two factors must be determined experimentally.

Various experiments are described which were made on the perch, *Perca fluviatilis*, the wrasse, *Crenilabrus melops*, the rockling, *Onos mustela* and the dragonet, *Callionymus lyra*. The results showed that there was a relation between the relative size of the swimbladder and the change in the density of a fish when it is subjected to a reduction in pressure; that the bladder and body wall of the perch offer little resistance to the expansion of the bladder gas; and that the danger of the bladder wall rupturing might restrict the extent of rapid movements made by the perch.

Experiments on the restriction that the swimbladder imposes to the rapid and slow vertical movements of the perch will be described in a following paper.

My thanks are due to Prof. A. J. Grove, under whose supervision the work was carried out. I am grateful to Dr G. Chapman and other friends for help with the typescript and to the Director and staff of both the Freshwater and the Marine Biological Associations for advice and assistance. Dr C. W. Kilmister kindly helped with the mathematics. Finally, I must thank Dr A. G. Lowndes for advice about the density experiments. I am grateful to London University, Queen Mary College and the D.S.I.R. for financial support while the work was in progress.

REFERENCES

- ALLEN, K. R. (1935). The food and migration of the perch (*Perca fluviatilis*) in Windermere. *J. Anim. Ecol.* **4**, 264-73.
- BODEN, B. (1950). Plankton organisms in the deep scattering layer. *Rep. U.S. Navy Electronics Lab.* no. 186.
- CHAPMAN, W. M. (1947). The wealth of the oceans. *Sci. Mon., N.Y.*, **66**, 192-7.
- DIETZ, R. S. (1948). Deep scattering layer in the Pacific and Antarctic Oceans. *J. Mar. Res.* **7**, 430-42.
- GRAHAM, M. (1924). The annual cycle in the life of the mature cod in the North Sea. *Fish Invest.* ser. 2, **6**, no. 6.
- HASLER, A. D. & BARDACH, J. E. (1949). Daily migrations of perch in Lake Mendota, Wisconsin. *J. Wildl. Mgt.* **13**, 40-51.
- HERSEY, J. B. & MOORE, H. B. (1948). Progress report on scattering layer observations in the Atlantic Ocean. *Trans. Amer. Geophys. Un.* **29**, 341-54.
- HICKLING, C. F. (1925). Notes on euphausiids. *J. Mar. Biol. Ass. U.K.* **13**, 735-45.
- HICKLING, C. F. (1927). The natural history of the hake. Parts I & II. *Fish Invest.* ser. 2, **10**, no. 2.
- JACOBS, W. (1933). Untersuchungen zur Physiologie der Schwimmblase der Fische. II. Die Volum-regulation in der Schwimmblase des Flussbarsches. *Z. vergl. Physiol.* **18**, 125-56.
- JOHANSEN, A. C. (1925). On the diurnal vertical movements of the young of some fishes in Danish waters. *Medd. Komm. Havundersøg., Kbh.*, Fiskeri, **8**, no. 2.
- JOHNSON, M. W. (1948). Sound as a tool in marine ecology, from data on biological noises and the deep scattering layer. *J. Mar. Res.* **7**, 443-58.
- JONES, F. R. H. (1949). The teleostean swimbladder and vertical migration. *Nature, Lond.*, **164**, 847.

- LOWNDES, A. G. (1937). The density of some living aquatic organisms. *Proc. Linn. Soc. Lond.* 150th session, part 2, 62-73.
- LOWNDES, A. G. (1941). The displacement method of weighing living aquatic organisms. *J. Mar. Biol. Ass. U.K.* **25**, 555-74.
- MOORE, H. B. (1950). The relation between the scattering layer and the Euphausiacea. *Biol. Bull. Woods Hole*, **99**, 181-212.
- MOREAU, A. (1876). Recherches expérimentales sur les fonctions de la vessie natatoire. *Ann. Sci. nat. Zool.* ser. 6, **4**, 1-85.
- MURRAY, SIR J. & HJORT, J. (1912). *The Depths of the Oceans*. 821 pp. Macmillan, London.
- PEARSE, A. S. & ACHTENBERG, H. (1918). Habits of the yellow perch in Wisconsin Lakes. *Bull. U.S. Bur. Fish.* **36**, 294-366.
- PLATTNER, W. (1941). Etudes sur la fonction hydrostatique de la vessie natatoire des poissons. *Rev. suisse Zool.* **48**, 201-338.
- RUSSELL, F. S. (1928). Vertical distribution of Marine Macroplankton. VIII. Further observations on the diurnal behaviour of the pelagic young of teleostean fishes in the Plymouth Area. *J. Mar. Biol. Ass. U.K.* **15**, 829-50.
- SOUTHERN, R. & GARDINER, A. C. (1932). The diurnal migrations of the Crustacea of the plankton in Lough Derg. *Proc. R. Irish Acad. B*, **60**, 121-59.
- TÂNING, Å. VEDEL (1918). Mediterranean Scopelidae. *Rep. Danish Oceanogr. Exped. Medit.*, Biol. **2**, A, 7.
- TAYLOR, H. F. (1921). Airbladder and specific gravity. *Bull. U.S. Bur. Fish.* **38**, 121-6.

FRAGMENTATION OF RED CELL GHOSTS IN RELATION TO THE PROBLEM OF RED CELL STRUCTURE

BY ERIC PONDER

Nassau Hospital, Mineola, N. Y.

(Received 23 April 1951)

(With One Text-figure)

An indication as to whether a body is a solid or a hollow shell can be obtained by dividing it into a number of fragments and seeing whether the total volume of the fragments is substantially the same as, or smaller than, the initial volume of the body. If the former is the case, the indication is that the body is a solid, and if the latter is the case, the indication is that the body is a hollow shell. This test can be applied to the human red cell and to the human red cell ghost by heating them to temperatures between 49 and 54° C., a treatment which produces fragmentation into large enough fragments to be counted and to be thrown down by centrifuging, always provided that the cells and ghosts are discoidal (Ponder 1950a).

METHODS

(1) *Preparation of discoidal ghosts*

Either of two methods is satisfactory. In the first, 1 vol. of a suspension, in 1% NaCl, of washed human red cells from fresh heparinized blood is added to 4 vol. of water; the volume concentration of the cells in the suspension should be at least 0.8. After standing for about 30 min. at 25° C., 1 vol. of 0.5 M- Na_2HPO_4 is added with rapid stirring. This produces 'reversal of haemolysis'. The ghosts are nearly all perfect, thin, disks which can easily be thrown down by centrifuging. Most of the supernatant fluid is removed, and the ghosts are resuspended in what is left to make a suspension with a volume concentration of between 0.25 and 0.35. Suspensions with a higher volume concentration are unsatisfactory to work with because, after heating, a fragment-free supernatant fluid is very difficult to obtain. The procedure can obviously be modified by using larger quantities of water to produce haemolysis, and larger quantities of greater concentrations of Na_2HPO_4 for bringing about reversal. NaCl can be used in place of Na_2HPO_4 , but the ghosts which result are apt to be less perfect disks.

In the second method, about 50 ml. of a suspension of washed cells with a volume concentration of about 0.1 are kept at 37° C. for 6–10 hr. The cells are then packed for 30 min. at 4000 r.p.m., and 4 ml. of the packed mass is added to 500 ml. of water. The mixture is allowed to stand for 24 hr. at 4° C., by the end of which time fluffy ghosts appear as a sedimented layer. Most of the supernatant fluid is decanted, and the remaining fluid with the layer of ghosts is centrifuged for 30 min. at about 4000 r.p.m. The ghosts are resuspended in a little of the supernatant fluid, and the

volume concentration of the suspension is measured by conductivity, an approximate value only being required. The volume concentration should be about 0.5. Sufficient 0.5 M- Na_2HPO_4 is added to restore isotonicity; again, an approximate restoration is all that is needed. This results in a shrinking of the ghosts,* and the final suspension usually has a volume concentration of between 0.25 and 0.35, which is satisfactory. These ghosts are discoidal and contain much less Hb than do the ghosts obtained by the first method.

(2) Fragmentation and measurement of volume

Fragmentation of the ghosts is produced by heating the suspension obtained by either of these two methods to between 49 and 54° C. for 3 min. These temperatures and times of heating fragment the discoidal ghosts into an average of from two to five fragments, not too small to be thrown down by centrifuging. The number of ghosts and of fragments is counted as already described (Ponder, 1950a).

The volume concentration of the suspension, before and after the fragmentation by heat, is found by a conductivity method (Fricke & Curtis, 1935; Ponder, 1935, 1942, 1950b), a small conductivity cell which requires only 0.3 ml. to fill it being used in conjunction with a conductivity and capacity bridge. To find the volume concentration ρ_g of the ghosts, we calculate

$$\rho_g = \frac{r_1/r_2 - 1}{r_1/r_2 + 1/X_g},$$

and compare it with the volume concentration for the fragments

$$\rho_f = \frac{r_3/r_4 - 1}{r_3/r_4 + 1/X_f}.$$

This supposes that the resistance of the ghosts is high in relation to the resistance of the surrounding fluid (Fricke & Curtis, 1935), and that the fragments are as non-conducting as the ghosts. There seems no reason to doubt either of these assumptions, and it should be noticed that if the fragments were more conducting than the ghosts, the value of ρ_f would be *underestimated*.

There is no difficulty in finding r_1 and r_2 , the resistances of the unheated suspension of ghosts and of the fluid surrounding them, for the fluid can be obtained essentially free from ghosts by centrifuging for 30 min. at a speed which gives about $2 \times 10^4 G$. Filtration (see below) does not increase the conductivity more than a few per cent. Nor is there any difficulty in measuring r_3 , the resistance of the heated and fragmented suspension of ghosts. The difficulty lies in the obtaining of the fluid surrounding the fragments for the measurement of the resistance r_4 . Centrifuging for 30 min. at $2 \times 10^4 G$. may be sufficient when the degree of fragmentation is small (such as occurs, for example, when the ghosts are heated to 50° C. for 3 min.), but when the degree of fragmentation is greater the fluid obtained by centrifuging tends to contain many small fragments, some approaching the size of myelin forms. These

* If the tonicity is not restored, it is remarkable that the ghosts can be heated, either in the water-bath or in the heating chamber (see Ponder, 1948, for description) to the temperature of the coagulation of Hb (about 70° C.) without fragmentation taking place.

have to be removed by filtration. Seitz micro-filters fitted with EK filter pads* are suitable for filtering a few ml. with suction, and the filtration can be made much more rapid, apparently without loss in efficiency, by cutting each pad into two pads of half-thickness. Fluid obtained in this way from a heated suspension of fragments has a resistance r_4 within 5% of the resistance r_2 of the fluid from the unheated suspension of ghosts. The heating of the latter fluid to temperatures as high as 55° C. has no appreciable effect on the resistance r_2 .

Examination of the unheated and heated suspensions shows that the ghosts in the former are excellent disks. Their volume is about 0.5 that of the intact cell (Ponder, 1950b)†, and their diameter to thickness ratio is about 6 to 1 at most. A value of 1.0 has been assigned to X_g ; if X_g is overestimated, as it probably is, the values of ρ_g appear larger than they really are. After heating, the fragments are spheres, so X_f is 2.0. It should be noticed that any failure to free the supernatant fluid from fragments will lead to an underestimation of ρ_f , and that any increase in the conductance of the fragments will affect the result in the same direction. The errors attached to the method are thus such as tend to make the value of the ratio ρ_f/ρ_g a *minimum* value.

RESULTS

Table 1 shows value of ρ_g and of ρ_f obtained in experiments in which the degree of fragmentation f (number of fragments per mm.³ after heating/number of ghosts per mm.³ before heating) varied from 1.8 to 5.7.

Table 1

Method	Vol. H ₂ O	Temp. (° C.)	f	ρ_g	ρ_f	ρ_f/ρ_g
I	4	49.0	1.8	0.29	0.25	0.81
2	—	49.5	2.2	0.25	0.20	0.80
I	15	51.0	2.7	0.17	0.15	0.88
I	6	52.0	4.3	0.25	0.22	0.88
2	—	52.0	3.9	0.56	0.47	0.84
I	4	53.0	5.7	0.33	0.31	0.94
2	—	54.0	5.1	0.26	0.22	0.81

The total volume of the fragments is over four-fifths that of the volume of the ghosts from which they are derived, and so the indication is that the ghost is a solid rather than a hollow shell. Provided that the temperature is high enough to produce considerable fragmentation (e.g. $f=2$) without there being much haemolysis, the

* Obtained from allergy supply houses (Allergists Supply Co., New York City). The filter pads should be thoroughly washed by sucking water through them, and dried before being used. Care must be taken to use a type of pad which does not produce a large pH change in the material passed through it.

† Earlier measurements (Ponder, 1942) led to the conclusion that the volume of the watery ghost (rabbit) is 'substantially the same' as that of the intact red cell from which it is derived. Quite often it was found to be smaller, but the emphasis was placed on the similarity rather than on the differences, because similarity in volume was such an unexpected observation at the time. The 1950 determinations, which show the volume of the rabbit ghost to be about 0.6 that of the intact cell, and the volume of the human ghost to be about 0.5 that of the intact cell, are much better than those of 1942. This is partly because they are less affected by stirring errors, which are almost absent from the small conductivity cells now in use, and partly because of greater familiarity with the material.

same result is found if the volume of the fragments into which human red cells break is compared with the volume of the cells themselves.*

Both the volume of the individual ghost and its Hb content depend on the quantity of water used to produce haemolysis; the volume must be partly determined by the amount of contained Hb, and it is likely that Hb is a component part of the structure (see below; and cf. Mitcheson, 1950; Moskowitz, Danliker, Calvin & Evans, 1950). Fragmentation as solid bodies, i.e. with a ρ_f/ρ_a ratio approaching unity, is characteristic of ghosts prepared with 4 vol. of water, with 15 vol. of water, or by method 2 in which the volume of water is very large. In these different methods of preparation, the volume of the ghost varies from about one-half to one-fifth, and the Hb content of the ghost from about one-third to less than one-fifteenth, that of the intact red cell, and so the fragmentation process does not seem to depend, at least primarily and in a simple way, on the content of Hb. Nor is it to be accounted for on the basis of Hb coagulation, for the temperatures at which fragmentation occurs are at least 15° C. below that at which denaturation of Hb begins.

DISCUSSION

What is already known about the structure of the red cell and its ghost (for a recent summary see Ponder, 1949) may now be restated so as to take account of the following observations: (1) that the ratio of the volume of the ghost to the volume of the red cell is very variable, and dependent on the way in which the ghost is prepared (Ponder, 1950*b*); (2) that the ratio of the Hb concentration in the ghost to that in the surrounding fluid is also variable, dependent on the method of preparation, and usually greater than 1.0 (Ponder, 1942, 1950*b*); and (3) that both red cells and ghosts fragment as if they were solid bodies rather than hollow shells. Two deductions from these observations seem inescapable. Because of its fragmentation as a solid body, the red cell ghost as prepared by the foregoing methods must be a relatively substantial object and not a bag surrounded by a membrane only a few hundred Ångströms thick; because of the variable volume and Hb concentration in the ghost, the object which is called the ghost must be variable in composition. These considerations lead to a picture of the structure of the red cell and of the ghost which is best approached by restating what is known about the nature and orientation of the materials which compose them.

It is certain that a surface ultrastructure gives rise to optical polarization phenomena (Schmitt, Bear & Ponder, 1936; Mitcheson, 1950) which are dependent

* If the temperature is higher and the degree of fragmentation greater, complexities are introduced because of haemolysis and the fragmentation of ghosts which are not themselves of negligible volume. Instead of the type of situation shown in Fig. 1, 1*a* (Ponder, 1950*a*) one gets the situation of Fig. 1, 4*a* or 6*a*. The distinction between the two situations might not be discoverable by experiments in which volume is measured with the haematocrit, because the viscosity of the system becomes so great as lysis nears completion that ghosts and fragments of ghosts become increasingly difficult to throw down. This difficulty was not foreseen, and is an instance in which the attempt at experimental illustration of the various possible cases has been misleading. If volumes were always determined by conductivity, most systems in which fragmentation is produced by heat would probably turn out to be examples of cases 5*a* and 6*a*, rather than 1*a* and 5*a*. Case 1*a* is probably a limiting case which would apply only to ghosts of the most attenuated variety.

on the thickness of the structure, the materials in it, and their orientations. The optical phenomena were accounted for at the time (1936) as due to a tangential arrangement of proteins and a radial arrangement of lipids, the thickness of the whole ultrastructure being much greater than the 30 Å. which Fricke, Danielli, and others had suggested on the basis of impedance measurements. The description may now be modified by suggesting that the tangentially arranged component is the lipo-protein elenin, the long fibres of which are joined by ether-extractable lipids to form a network (Moskowitz *et al.* 1950; Danliker, Moskowitz, Zimm & Calvin, 1950), which, together with Hb and perhaps another protein called S-protein, makes up the surface ultrastructure of the cell and ghost. Elenin, deprived of its alcohol-extractable lipids, may be the same as the stromatin of Jorpes (1932); a network can certainly be seen in electron microscope photographs of ghosts (Bessis & Bricka, 1949) and also in electron microscope photographs of elenin itself (Danliker *et al.* 1950). This description supplies a tangential component, but is non-committal about a radial one. Mitcheson's view is that the negative intrinsic birefringence and the positive form birefringence are both supplied by bundles of protein which lie in the plane of the surface but which are looped with the loops lying radially. This description does not place much weight on the type of evidence which led Schmitt *et al.* to distinguish between lipid and protein components, viz. the changes in birefringence which take place when the ghost is extracted with lipid solvents, suspended in media of high refractive index, etc.; if the surface ultrastructure is made up of lipoprotein complexes, however, this evidence may be worth less than it was thought to be when it was obtained. Some objection can be made to Mitcheson's results because they were obtained with ghosts in glycerine, but it seems probable that similar values as regards the order of magnitude of the thickness will be found for ghosts in saline. Still other possible arrangements of the components of the red cell surface ultrastructure are discussed by Frey-Wyssling (1948), and it is impossible to decide between the several alternatives at the present time. What is under discussion, however, is clearly a complex and highly oriented structure, with little resemblance to the over-simplified models of fifteen years ago.

Two other properties of the red cell surface ultrastructure, its thickness and the extent to which Hb is associated with it, are inseparable from each other, for if the structure has more than a small thickness, it must contain Hb, and vice versa. A more immediate question is whether the surface ultrastructure is to be thought of as constant or variable. The evidence certainly points towards variability (variable volume and Hb content of the ghost, the fragmentation at the same time being that of a solid body). This variability will be likely if the distinction between the surface ultrastructure and the interior of the red cell is largely a matter of how the ghost is prepared, and especially if there is an ultrastructure in the interior as well as at the surface. The evidence for an internal ultrastructure has been summarized already (Ponder, 1949), but special weight ought to be placed on the direct evidence provided by the X-ray diffraction patterns of Dervichian, Fournet & Guinier (1947); these show an arrangement of the Hb molecules in the red cell interior of the type

which occurs in the 'expanded crystal' i.e. intermediate between the order of the crystal and the disorder of the dilute solution.

The relation between the highly ordered ultrastructure and the less ordered, but yet not randomly arranged, interior can be represented by a diagram (Fig. 1). The abscissa shows distances in microns along a cross-section of a quadrant of a red cell, reckoning from the centre *O*. The upper graph represents the concentration of Hb and of lipoprotein (or of the lipids plus the proteins other than Hb which make

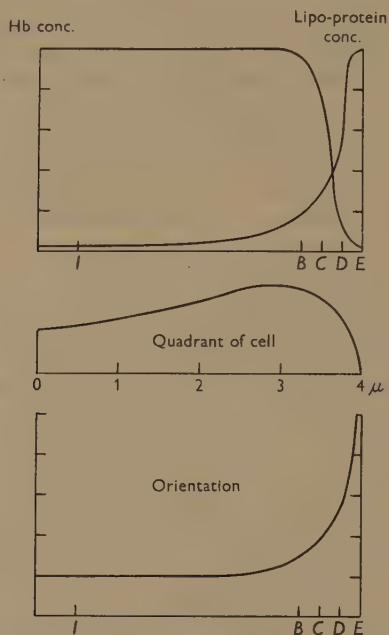


Fig. 1. To illustrate the structure proposed for the human red cell and ghost. The diagram of the quadrant of the cross-section of the cell supplies the scale of the abscissae; the scales on the ordinates are arbitrary. For description, see text.

up the surface ultrastructure). The former is shown on the left ordinate and the latter on the right ordinate; since the lipoprotein concentration is of the same order of magnitude as that of the Hb when both are expressed in mols., the ordinates can be made of equal size. The concentration of lipoprotein is shown as very great at the surface *E* and very small in the interior *I*; the concentration of Hb is large in the interior *I* and small, but not zero, near the surface *E*. Both concentrations are shown as falling continuously from their high values to their low ones, the fall being steep in the region of *C*, about 0.5μ in from the surface *E*. The value 0.5μ is chosen because a ghost with walls of this thickness collapsed on each other would occupy about half the volume of the red cell, the value found experimentally when ghosts

are prepared by the methods used here. Towards the surface, e.g. at *D*, the structure is predominantly lipoprotein with some Hb contained in it. The Hb molecules in this region are probably oriented with respect to the oriented lipoproteins,* so that the Hb molecules form part of the ultrastructure (Ponder, 1942; Mitcheson, 1950; Moskowitz *et al.* 1950). Towards the interior, e.g. at *B*, the structure is preponderantly Hb, but the Hb molecules of this region are probably still oriented, to some degree, with respect to the lipoproteins which are present in the region *BCD*. The concentration of the lipoprotein is to be thought of as falling as one goes inwards, and as it does so the orientation of the associated Hb molecules will become less, so that in the interior at *I* the only orientation left is the short-order arrangement of the expanded crystal of Hb.

The purpose of the lower part of Fig. 1 is to convey diagrammatically that the degree of orientation of the components which make up different regions of the red cell is high at the surface *E* and that it falls sharply in the region *BCD*, some $0.25\text{--}0.5\mu$ from the surface, to the level of the orientation of Hb in the interior. This level is not zero, and the fall from the high level at *E*, while steep, involves no discontinuity. Different methods of preparation can accordingly result in ghosts of different volumes, Hb contents, and compositions. In the forms of lysis which produce the least injury, the poorly oriented Hb at *I* will pass into solution, particularly if water has entered the cell during a preliminary swelling process, and what will be left will be the relatively thick Hb-containing portion *BCDE*. The result will be a voluminous ghost with a large amount of Hb contained in it, not as pigment in a bag, but as part of its structure. Other forms of lysis may cause the structure to break down, not at *B*, but at *C* or even at *D*; the ghosts will then be thinner, less voluminous, and will contain less Hb. Washing will tend to reduce the thickness of any of these ghosts, so that after many washings there may be nothing left but the most highly oriented region at *E*; these attenuated ghosts will have a negligible volume and a negligible Hb content, and seem to be the ghosts which are found when very large volumes of water are used to bring about haemolysis (Parpart, 1931), when large volumes are used for repeated washing, and in systems containing large concentrations of various lysins (Ponder, 1950*b*).

A feature of this suggested structure of the red cell as a body composed of molecules which are oriented in different degrees but throughout its entire structure is that the lipids of the cell and ghost are concentrated at the surface *E* and that the orientation at *E* is particularly high. There is no objection to the electrical resistance, the permeability properties, or even the biconcave shape depending principally on the material at *E*, nor to the thickness of the highly oriented layer at *E* being of the order of 100 Å. This, however, has little to do with the thickness or chemical composition of any ghost except one of an extremely attenuated type; it has also little to do with the polarization optics or the thickness and volumes of ghosts prepared by any

* The principle involved here is essentially the same as the one which applies to the situation in which there is short-order orientation (Dervichian, 1950), viz. if an asymmetrical molecule comes to lie in the neighbourhood of oriented molecules, all orientations of the asymmetrical molecule are not equally likely, and the molecule becomes oriented, if only in a statistical sense, with respect to its neighbours.

procedure except one which washes away or leaches out lipid and proteins, including Hb, from the ultrastructure. It follows, however, that the material which makes up the ghost must be, on the average, highly hydrated, since the ghosts prepared by methods such as those described above have volumes as great as half that of the red cell, fragment as solid bodies, and contain only from 2 to 4% of the solids of the red cell (*excluding* Hb) by weight (Fricke, Parker & Ponder, 1939). More specifically, the region *BCD* has to be thought of as a highly hydrated complex of lipoproteins, Hb, and probably other proteins in addition.

SUMMARY

Human red cell ghosts are prepared by methods which yield ghosts with a volume between one-half and one-fifth that of the intact red cell. These ghosts, when heated to temperatures between 49 and 54° C., break into a number of fragments whose total volume is approximately the same as that of the ghost before it fragmented. This indicates that the ghosts prepared by these methods are solid bodies rather than hollow shells.

The ghosts contain from one-third to one-fifteenth of the Hb of the intact red cell, probably as an integral part of their structure. This structure is discussed in relation to a proposed structure for the red cell itself. The evidence points to the red cell being composed of lipid, Hb and proteins other than Hb, to these being oriented to a decreasing, but never negligible, extent from without inwards, and to the properties of the ghost (volume, Hb content, chemical composition, etc.) being largely dependent on the way in which it is prepared.

REFERENCES

- BESSIS, M. & BRICKA, M. (1949). Nouvelles études sur les cellules sanguines au microscope électronique. *Arch. Anat. micr.* **38**, 190-215.
- DANLIKER, W. B., MOSKOWITZ, M., ZIMM, B. & CALVIN, M. (1950). The physical properties of elenin, a lipoprotein from human erythrocytes. *J. Amer. Chem. Soc.* **72**, 5587-92.
- DERVICHIAN, D. G. (1950). Dalla struttura dell' emoglobina alla struttura del globulo rosso. *Ann. Chim.* **40**, 300-9.
- DERVICHIAN, D. G., FOURNET, G. & GUINIER, A. (1947). Mise en évidence d'une structure submicroscopique dans les globules rouges par la diffusion des rayons X aux petits angles. *C.R. Acad. Sci., Paris*, **224**, 1848-50.
- FRANKLIN, A. E. & QUASTEL, J. H. (1950). Paper chromatograms of protein mixtures and blood plasmas. *Proc. Soc. Exp. Biol., N.Y.*, **74**, 803-8.
- FREY-WYSSLING, A. (1948). *Submicroscopic Morphology of Protoplasm and its Derivatives*. New York: Elsevier Publ. Co.
- FRICKE, H. & CURTIS, H. J. (1935). The electrical impedance of hemolysed suspensions of mammalian erythrocytes. *J. Gen. Physiol.* **18**, 821-36.
- FRICKE, H., PARKER, E. & PONDER, E. (1939). Relative quantity of the fixed framework of the hemolysed rabbit red cell. *J. Cell. Comp. Physiol.* **13**, 69-76.
- JORPES, E. (1932). The protein components of the erythrocyte membrane or stroma. *Biochem. J.* **26**, 1488-1503.
- MITCHESON, J. M. (1950). Thickness and structure of the membrane of the human red cell ghost. *Nature, Lond.*, **166**, 347-9.
- MOSKOWITZ, M., DANLIKER, W. B., CALVIN, M., and EVANS, R. S. (1950). Studies on the antigens of human red cells. I. The separation from human erythrocytes of a water soluble fraction containing the Rh, A, and B factors. *J. Immunol.* **65**, 383.
- PARPART, A. K. (1931). Is osmotic hemolysis an all or none phenomenon. *Biol. Bull. Woods Hole*, **61**, 500-17.

- PONDER, E. (1935). The measurement of red cell volume. Conductivity measurements. *J. Physiol.* **85**, 439-49.
- PONDER, E. (1942). On properties of the red cell ghost. *J. Exp. Biol.* **18**, 257-65.
- PONDER, E. (1948). Shape and shape transformations of heated human red cells. *J. Exp. Biol.* **26**, 35-45.
- PONDER, E. (1949). Lipid and protein components in the surface ultrastructure of the erythrocyte. *Faraday Discussion on Lipoproteins*, no. 6, pp. 152-60.
- PONDER, E. (1950*a*). A quantitative study of the fragmentation and haemolysis of mammalian red cells by heat. *J. Exp. Biol.* **27**, 198-209.
- PONDER, E. (1950*b*). Observations sur certaines propriétés des stromas de globules rouges. *Rev. Hemat.* **5**, 580-90.
- SCHMITT, F. O., BEAR, R. S. & PONDER, E. (1936). Optical properties of the red cell membrane. *J. cell. Comp. Physiol.* **9**, 89-92.

THE PHASES OF THE OESTROUS CYCLE IN THE ADULT WHITE RAT

By ANITA M. MANDL

Department of Anatomy, University of Birmingham

(Received 8 April 1951)

(With Two Text-figures)

Since the classical description of Long & Evans (1922) of the cyclical changes which take place in the vaginal smear of the rat, vaginal smear histology has always been used as an index of ovarian activity. It is also used as a test of the potency of various steroid hormones (e.g. Emmens, 1939), and as a means of correlating cyclical ovarian changes with those in the accessory reproductive organs and other endocrine organs (e.g. Astwood, 1939; Bourne & Zuckerman, 1940). Numerous studies have been made of the cytology of shed vaginal cells by means of trichrome stains (e.g. Hartman, 1944), and various modifications have been suggested for the original technique proposed for taking vaginal smears.

Under normal laboratory conditions it is inconvenient to take smears more than once a day. At the same time it is known that frequent smearing may itself induce cornification (Astwood, 1939). In view of the need to time various operative procedures accurately in relation to the phases of the oestrous cycle, the following study was undertaken (*a*) to determine the individual variability of cycle length in our own colony of rats and (*b*) to compare our data with those reported for other laboratories.

MATERIALS AND METHODS

Animals

Observations were made on thirty-nine adult virgin females derived from ten litters belonging to an inbred colony of albino rats. They were 98–186 days old at the start of the experiment. Each litter-group was housed in a separate cage, and supplied with food and water *ad lib*.

Experimental

Daily vaginal smears were taken for 83–88 days, using a loop of nickel-chromium wire heated in a flame and cooled in saline. The smears were fixed in a modified Schaudinn's solution, consisting of equal volumes of absolute alcohol and saturated mercuric chloride solution. After rinsing in 90% alcohol containing a few drops of iodine in potassium iodide, and then in water, the preparations were stained in Mann's eosin-blue solution.

To allow of more detailed histological examination, the following technique was developed. After the usual fixation, the smears were stained for 5 min. in acidified 1% Ponceau-de-xylidine red solution, rinsed in distilled water acidified with acetic acid, and then differentiated in 1% phosphomolybdic acid. They were then counter-

stained in 2-5% methylene blue for 1 min. and rinsed in tap water. The smears were either examined wet, or dehydrated, cleared and mounted in Canada balsam. This technique stains acidophil cytoplasm red, and basophil cytoplasm blue. Although the differentiation is not as clear as with trichrome stains (Hartman, 1944; Shorr, 1941), the method is much more rapid and can be used for routine purposes.

All the stained smears were stored and subsequently re-examined in order to check the diagnosis of the following six stages of the cycle, based on published classifications (e.g. Astwood, 1939):

Early dioestrus: thick smear consisting almost entirely of leucocytes; some cornified cells, Shorr cells and a few basophilic epithelial cells.

Dioestrus: thin smear, consisting of leucocytes, basophilic epithelial cells undergoing vacuolation, practically no cornified cells, diminishing numbers of Shorr cells, and Hartman's type V 'phagocytozed' cells.

Late dioestrus: leucocytes, some vacuolated and some clearly nucleated basophilic epithelial cells, no cornified cells, no Shorr cells.

Early oestrus: thick smear consisting of large numbers of hexagonal basophilic nucleated epithelial cells, often disposed in a honeycomb pattern. Towards the end of this phase, the cells separate and cornification sets in.

Oestrus: all, or almost all, the epithelial cells have lost their nuclei and have become cornified. Towards the end of this phase, the smear becomes 'cheesy'.

Late oestrus: the smear is similar to the oestrous smear, but, in addition to many cornified cells, there are some large basophilic Shorr cells, and some small basophilic epithelial cells. As soon as the leucocytes appear, late oestrus merges into early dioestrus.

Smears were often diagnosed as being intermediate between two of the above stages, and thus twelve were recorded (e.g. L.D./E.O., L.O./E.D., etc.).

For a check on diagnosis, 1100 smears were selected at random and re-examined, and the two sets of data compared.

About a fortnight before autopsy, the mean length of the cycle was calculated for each animal, and the date at which it would be at a preselected stage of the cycle estimated. Starting 3 days before autopsy, smears were taken twice daily, as nearly as possible at 12-hourly intervals, so that the precise stage at which the animals were killed was known. As the greatest changes in the accessory reproductive organs are known to occur between late dioestrus and oestrus (Astwood, 1939), twenty-eight out of the thirty-nine animals were killed within this period. The times of autopsy of the remaining eleven animals were distributed over the other stages of the cycle.

When all the smear records were complete, the mean interval between successive phases of oestrus (i.e. the cycle length) was calculated for each animal. The relative lengths of the various stages were determined by Astwood's (1939) method. For each animal, the number of days on which each of the six stages of the cycle was recorded was expressed as a percentage of the total period of observation (i.e. 83-88 days). The mean cycle length (in hours) for each individual rat was then subdivided into its six phases according to the ratio of these percentages. Means for litter-groups and a weighted mean for the whole series were then calculated.

Autopsy

The animals were weighed and killed with chloroform within 5 min. of the final smear. The ovaries, adrenals and uteri were dissected out and weighed before fixation. The uteri were weighed twice—first with the fluid in the lumen, and then after the fluid had been drained.

Statistical analysis

Standard statistical procedures (Fisher, 1946) were used. Comparisons were made by *t*-tests, by the χ^2 test and by analyses of variance.

RESULTS

Regularly recurring oestrous cycles were observed in thirty-one of the thirty-nine animals. In the remaining eight animals, oestrous cycles occurred for only part of the time, and were interrupted by periods of dioestrus which varied between 11 and 60 days. Leucocytes were rarely absent from the vaginal smears of these animals. Subsequent observations showed that the irregularity of the oestrous cycles of these rats was due to some pathological condition of the reproductive organs. Those cycles in the eight irregular animals in which the period of dioestrus did not exceed 7 days were regarded as being within the normal range and were included in the analysis.

Histology

Daily smears relating to a total of 659 'normal' cycles (over 3000 smears) were examined. The preparations showed all the cell types already described by Hartman (1944) and Jaworski (1950).

Statistical analysis

(a) Cycle length. The mean duration for the 659 cycles was 4.4 ± 0.04 days. Analysis showed that the variance in cycle length is significantly less within than between individual rats ($P < 0.001$), both in the present series of data and in that published by Long & Evans (1922). Litter mates tended to cycle more consistently than unrelated rats ($P \approx 0.05$). There was no significant difference in cycle length within as compared to between litter-mates in eight out of the ten litters (Table 1).

The mean cycle length of the present series (4.4 ± 0.04 days) is significantly shorter than that computed from Long & Evans' data (5.6 ± 0.08 days; $P < 0.001$). The mode, however, is 4 days in both sets of observations. The two colonies differ in so far as the frequency cycles of 4 days and less is significantly higher in our series ($P < 0.001$); the frequency is significantly lower above 4 days ($P < 0.001$). In both series the distribution around the mode is skew, the incidence of cycle lengths of more than 4 days being greater than for cycles of less than 4 days (Table 2).

(b) *Length of individual phases of the oestrous cycle.* Table 3 shows that the variance in the duration of dioestrus and late dioestrus is significantly less between litter-mates than between unrelated rats ($P < 0.001$). With the exception of late oestrus, which is on the border-line of statistical significance ($P = 0.01-0.01$), the remaining stages vary as much within, as between, litter-groups.

Table 1. *Length of oestrous cycle (in days) in thirty-nine adult rats, based on 659 oestrous cycles. Variance within and between litter-mates*

Litter no.	Mean cycle length in days for litter-groups	Mean cycle length in days for individual rats	P (analysis of variance)
1	4.5	4.0 4.1 4.0 5.9 4.4	<0.001
2	4.2	4.0 4.0 4.7	±0.5
3	4.1	4.2 4.0 3.9 4.2 4.1 4.4	0.2-0.1
4	4.7	4.5 5.4 4.1	0.2-0.1
5	4.3	4.0 4.1 4.3 4.5 4.5	>0.2
6	4.1	4.0 4.4 4.1	>0.2
7	4.1	4.2 4.0 4.0	>0.2
8	5.2	5.1 6.1 4.4 5.1	±0.05
9	5.0	4.3 4.7 5.7 5.4	0.01-0.001
10	4.3	4.6 4.1 4.1	0.2-0.1
Overall mean 4.4 ± 0.04 days			
Variance within and between litter-groups			±0.05
Variance within and between individual rats:			
Present series			<0.001
Long & Evans (1922)			<0.001

Table 2. *Frequency distribution of cycle lengths (in days) in present series of observations and as reported by Long & Evans (1922). Comparison of relative frequency of cycle lengths in the two series*

Cycle length (in days)	Relative frequency of cycle length in days		P (χ^2 test)
	Long & Evans (%)	Present series (%)	
1.5	—	0.2	Below 4 days, <0.001
2.0	0.4	0.2	
2.5	—	0.5	
3.0	3.3	4.0	
3.5	—	10.9	4 days, <0.001
4.0	38.2	49.9	
4.5	—	12.0	
5.0	30.4	11.7	
5.5	—	3.2	Above 4 days, <0.001
6.0	11.2	2.9	
6.5	—	1.2	
7.0	4.1	2.0	
7.5	—	—	
8.0	2.6	0.8	
8.5	—	0.3	
9.0	2.2	0.2	
Over 9	7.4	0.3	
Mean	5.6 days	4.4 days	
Mode	4 days	4 days	

This suggests that differences in the length of the whole cycle in different animals are due to variation in the duration of dioestrus, and that the duration of the oestrous phase is more or less constant.

Fig. 1 shows the relative duration of the phases of the cycle as reported by Long & Evans (1922), Astwood (1939), Jaworski (1950), and as determined in the present

Table 3. *Mean duration of the six phases of the oestrous cycle, and results of analyses of variance (variance within and between litter-groups)*

E.D. = early dioestrus E.O. = early oestrus
 D. = dioestrus O. = oestrus
 L.D. = late dioestrus L.O. = late oestrus

Stage	Overall mean duration (hr.)	Mean length of stage (hr.) for litter-groups Litter numbers										P analysis of variance)
		1	2	3	4	5	6	7	8	9	10	
E.D.	24	34	17	17	22	21	23	25	25	29	23	> 0.2
D.	28	22	23	28	31	23	29	24	44	32	28	< 0.001
L.D.	7	4	10	8	8	4	7	3	10	9	6	< 0.001
E.O.	18	19	15	17	14	12	11	20	21	22	15	0.1-0.05
O.	25	23	31	25	27	32	24	22	19	22	20	0.2-0.1
L.O.	5	5	6	4	8	4	5	4	5	3	10	0.05-0.01

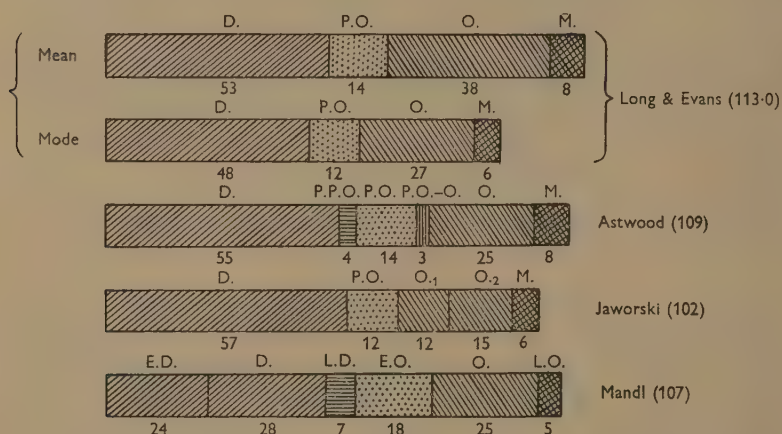


Fig. 1. The relative duration of the phases of the oestrous cycle. E.D. = early dioestrus; D. = dioestrus; L.D. = late dioestrus; E.O. = early oestrus; P.P.O. = pre-oestrus; P.O. = pro-oestrus; O. = oestrus; L.O. = late oestrus; M. = metoestrus.

investigation. The figures of Long & Evans and Jaworski are based on the study of smears taken every few hours, while those for the other two studies are indirectly based on the frequency with which different stages were observed in daily smears. It is clear that the relative duration of the different phases is very similar in the four series of data. Long & Evans observed that while the mean duration of oestrus is 38 hr., the mode is 27; the higher value of the mean is due to the fact that a number of their rats remained in oestrus for prolonged periods. In view of Astwood's (1939) later finding, it seems probable that the prolonged cornification noted by Long & Evans in some animals was due to too frequent smearing. It is also possible that the

colony included some rats in which persistent oestrus occurred spontaneously (Everett, 1939).

The difference between the present observations and those of Long & Evans in the duration of dioestrus, early oestrus and metoestrus were not statistically significant ($P=0.5-0.3$, $0.5-0.3$ and $0.7-0.5$ respectively).

(c) *Personal error.* Of 1100 smears selected at random and re-examined without reference to the previous recorded diagnosis, 653 diagnoses were in complete agreement; 244 differed by $\frac{1}{2}$ stage; 156 differed by 1 stage and 47 by $1\frac{1}{2}$ stages. There were no disagreements of 2 or more stages. Differences in diagnosis were inconsistent in direction so far as the stages of the cycle were concerned. The mean error was 0.3 stage per reading. It would probably have been smaller if reference had been made, for purposes of guidance, to the diagnosis of the preceding days' smears.

So far as we are aware, there is no previous published record of a systematic check on the accuracy of diagnosis of vaginal smears.

Body weight at autopsy

The animals were fully grown, and at autopsy ranged from 183 to 273 days in age. Body weights varied from 156 to 242 g. (mean 200 g.) and appeared not to bear any constant relation to age.

An analysis of variance showed that the variance in body weight was significantly less within than between litter-groups ($P \approx 0.001$). Variance in body weight within and between stages of the cycle was not significantly different ($P=0.2-0.1$). This suggests that body weight does not fluctuate consistently in phase with the oestrous cycle.

Ovarian weight

The mean ovarian weight for the thirty nine rats was 61.2 mg. (range 37-88.5 mg.). Five of the eight rats which had been in persistent dioestrus had small ovaries (38.5-47.0 mg.).

The variance in ovarian weight did not differ significantly within and between litter groups ($P \approx 0.1$), but the variance within litter-groups was the smaller.

The variance in ovarian weight in animals at the same stage of the cycle tended to be less than that in animals at different stages of the cycle ($P=0.1-0.05$). Rats killed between late dioestrus and oestrus had heavier ovaries (mean 62.9 mg.) than those killed at the remaining stages (mean 56.9 mg.). A plot of the frequency distributions showed that in animals killed between late dioestrus and oestrus, both the mean and the mode had shifted to the right, as compared with animals killed at other phases of the cycle. The difference in mean failed, however, to reach statistical significance.

The variance between the right and left ovaries of the same individual did not differ significantly from that between right and left ovaries of rats at the same stage of the cycle ($P=0.2-0.1$), but it was significantly less than that between litter-mates and between rats at different stages of the cycle ($P < 0.001$).

Adrenal weights

The mean adrenal weight for the series was 65.1 mg. (range 40-94 mg.). Three of the eight animals that had been in persistent dioestrus had low adrenal weights (40, 50 and 50.5 mg.).

It has been shown by Andersen & Kennedy (1932) and Bourne & Zuckerman (1940) that the adrenal glands are heavier during oestrus than at other times. A comparison of the means of the weights recorded in the present study showed again that the glands tended to be heavier between late dioestrus/early oestrus and oestrus than at other times; the differences, however, were not significant statistically ($P=0.1-0.5$). A plot of the frequency distributions of adrenal weights during the oestrous phase compared to those in other phases showed that both the mean and the mode of the adrenal weights have shifted to the right at oestrus.

An analysis of variance, on the other hand, showed that the variance in adrenal weight did not differ significantly within and between stages of the cycle ($P=0.1-0.05$). This result, like the corresponding one on the ovary, is not, however, inconsistent with the thesis that the weights of the ovaries and adrenals vary in phase with the oestrous cycle.

Further analyses of variance showed that the variance in adrenal weight did not differ significantly within and between litter-groups ($P=0.1-0.05$); the variance between right and left adrenals of the same individuals was significantly less than that within and between groups of animals at the same stage of the cycle, and within and between litter-groups ($P<0.001$).

Uterine weight

The weight of the uterus ranged from 206 to 1370 mg. before any fluid was drained from the lumen, and from 206 to 760 mg. after draining. The two lowest uterine weights (206 and 223 mg.) were recorded in animals that had been in persistent dioestrus.

The manner in which the uterine weight varies in phase with the oestrous cycle is shown in Fig. 2. When the uteri were weighed with the fluid in the lumen, the variance within stages of the cycle was significantly lower than that between stages ($P=0.01-0.001$); the difference was not significant after the fluid had been drained ($P=0.2-0.1$). Fig. 2 shows however, that the weight of the uterus without fluid is higher at oestrus than at other times. This observation agrees with previous reports (e.g. Astwood, 1939).

In both analyses, variance in uterine weight did not differ significantly within and between litter-groups ($P>0.2$).

DISCUSSION

The analysis of the data collected in the present study, as well as that of Long & Evans (1922), indicates that variation in the length of the oestrous cycle in the adult white rat is greatly influenced by the genetic make-up of the individual. It also shows that the duration of oestrus is much more constant than that of dioestrus, which

consequently has a greater influence on the variability of the whole cycle. These findings point to the advantage of determining the mean cycle length of individual rats before they are submitted to experimental procedures designed to elucidate some aspect of reproductive physiology.

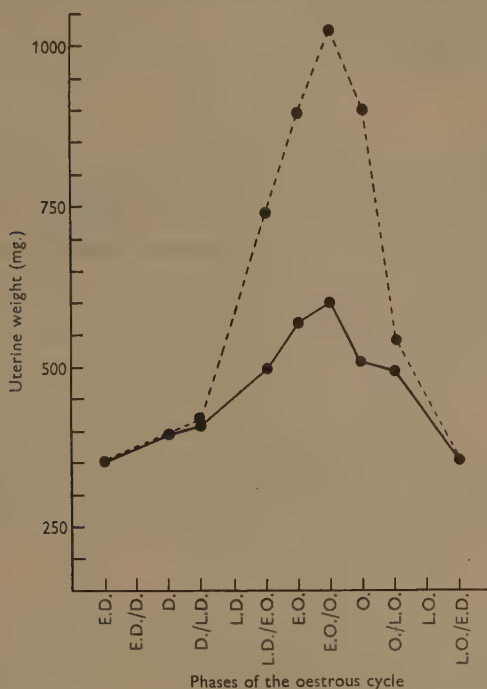


Fig. 2. Uterine weight in relation to the oestrous cycle. --- uterine weight including fluid in lumen; — uterine weight after drainage of fluid in lumen.

Apart from these new observations, and the check made on the reliability of the diagnosis of vaginal smears, the present study confirms the results previously published by Long & Evans (1922), Astwood (1939), Hartman (1944) and Jaworski (1950).

SUMMARY

The duration of the oestrous cycle in thirty-nine adult rats derived from ten litters was 4.4 ± 0.04 days.

The variance in cycle length tended to be less within than between litter-groups ($P \approx 0.05$). It was significantly less for individual rats than between individuals ($P < 0.001$).

The mean duration of early oestrus was 18 hr., oestrus 25 hr., late oestrus 5 hr., early dioestrus 24 hr., dioestrus 28 hr. and late dioestrus 7 hr. The length of the

dioestrous phase is variable and determines the length of the cycle, while the duration of oestrus appears to be relatively constant.

The figures have been compared with those published by other laboratories.

Analyses of variance did not disclose any significant fluctuation in the weight of the body, adrenals or ovaries in phase with the oestrous cycle. The uterus showed a characteristic increase in weight immediately before the onset of oestrus.

The author wishes to thank Prof. S. Zuckerman for his valuable help and advice.

REFERENCES

- ANDERSEN, D. H. & KENNEDY, H. S. (1932). Studies on the physiology of reproduction. IV. Changes in the adrenal gland of the female rat associated with the oestrous cycle. *J. Physiol.* **76**, 247-60.
- ASTWOOD, E. B. (1939). Changes in the weight and water content of the uterus of the normal adult rat. *Amer. J. Physiol.* **126**, 162-70.
- BOURNE, G. & ZUCKERMAN, S. (1940). Changes in the adrenals in relation to the normal and artificial threshold oestrous cycle in the rat. *J. Endocrinol.* **2**, 283-310.
- EMMENS, C. W. (1939). Variables affecting the estimation of androgenic and oestrogenic activity. *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 234, pp. 5-71.
- EVERETT, J. W. (1939). Spontaneous persistent estrus in a strain of albino rats. *Endocrinology*, **25**, 123-7.
- FISHER, R. A. (1946). *Statistical Methods for Research Workers*, 10th ed. London: Oliver and Boyd.
- HARTMAN, C. G. (1944). Some new observations on the vaginal smear of the rat. *Yale J. Biol. Med.* **17**, 99-111.
- JAWORSKI, Z. (1950). Aspects nucléaires et affinités tinctoriales cytoplasmiques des frottis vaginaux chez la rate. Etude comparative du cycle normal de la maturation sexuelle et du cycle artificiel chez l'animal castré. *Ann. Endocrinol.* **11**, 361-88.
- LONG, J. A. & EVANS, H. M. (1922). The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. Calif.* **6**, 1-148.
- SHORR, E. (1941). A new technic for staining vaginal smears. III. A single differential stain. *Science*, **94**, 545-6.

CYCLICAL CHANGES IN THE VAGINAL SMEAR OF ADULT OVARIECTOMIZED RATS

By ANITA M. MANDL

Department of Anatomy, University of Birmingham

(Received 8 April 1951)

(With Four Text-figures)

According to Kostitch & Telébakovitch (1929), cyclical changes continue to occur in the vaginal smear of rats after removal of both ovaries. The following study was undertaken because of the important bearing this observation has on an understanding of the rhythmical changes which occur in the accessory reproductive organs, and because no reports of its confirmation have appeared in the literature.

MATERIALS AND METHODS

Animals

Observations were made on twenty-six adult females derived from six litters belonging to an inbred colony of albino rats. At the time of operation, they ranged from 71 to 119 days in age, and from 103 to 236 g. in weight. Each litter-group was housed in a separate cage and supplied with food and water *ad lib*.

Experimental

The animals were ultimately required for a study of the influence of oestrogen on the uterine artery, and to this end all were unilaterally hysterectomized (Mandl & Zuckerman, 1951*a*). Nineteen were also bilaterally ovariectomized. The remaining seven were left as controls. The arrangement of the experiment is shown in Table 1.

Table 1. *Arrangement of experiment*

Litter no.	Mean body weight (g.) at operation	Age at operation (days)	No. of rats	No. of rats ovariectomized
1	215	118	6	4
2	178	119	2	2
3	165	92	4	3
4	133	74	4	3
5	170	72	2	1
6	124	71	8	6
Total			26	19

The animals were weighed and anaesthetized by means of Avertin, using a dose of 0.8 c.c. of a freshly prepared 2½% solution in warm saline per 100 g. body weight. The right horn of the uterus was removed through a ventral abdominal

incision by means of an electro-cautery needle, without the use of an earthing plate (Mandl & Zuckerman, 1951*b*). Care was taken not to damage the main uterine vessels. The ovaries were removed through the usual dorso-lumbar incision. The branches of the artery to the ovary and the Fallopian tube were divided by means of the cautery, care being taken not to injure the branch connecting the ovarian with the uterine artery. The ovaries were then isolated by cauterizing the hilus and excised with fine scissors. The wounds were closed in the usual way.

Vaginal smears were taken daily for 66–67 days after the operation. The smears were fixed and stained by the method already described (Mandl, 1951), and classified into the following six stages: early dioestrus, dioestrus, late dioestrus, 'early oestrus', 'oestrus', and 'late oestrus'. In addition, the presence of mucus, of sepsis

Table 2. *Duration of the oestrous cycle and its oestrous and dioestrous phases in spayed and control animals*

E.D. = early dioestrus E.O. = early oestrus
D. = dioestrus O. = oestrus
L.D. = late dioestrus L.O. = late oestrus

	Spayed (hr.)	Control (hr.)	P
Length of oestrous phases (E.O., O. and L.O.)	33	46	0.01–0.001
Length of dioestrous phases (E.D., D. and L.D.)	81	61	0.2–0.1
Length of whole cycle	114	107	0.7–0.6

and of any other unusual feature was noted. Owing to the dryness of the vagina after ovariectomy, aggregates of cells resembling those of the vaginal wall were occasionally present in the smear, in spite of the fact that great care was taken not to damage the vaginal lining.

All the stained preparations were stored for a subsequent check of the reliability of diagnosis.

Statistical analysis

The duration of the whole cycle and of the dioestrous and oestrous phases were compared with those of control animals by standard statistical procedures (Fisher, 1946).

Personal error

One hundred smears were selected at random and re-diagnosed without reference to the record of the smears of the preceding day. The mean difference between the two sets of readings was then calculated.

Autopsy

A careful search was made at autopsy of spayed animals for any remaining fragment of ovarian tissue. The adrenal glands and the uterine horn were weighed.

RESULTS

All animals rapidly recovered from the operation. They gained weight, and at the time of autopsy, 6-10 days after the end of the experimental period of 66-67 days their weights ranged from 156 to 308 g. (mean 229 g.). The spayed animals tended to weigh more than the controls, but the difference was not significant statistically.

No ovarian tissue was macroscopically visible at autopsy in any spayed animal. The uterine horn was thread-like, and the adrenals small.

Vaginal smears

The vaginal smear of the spayed rat differs from that of the normal animal in the consistent presence of leucocytes, in the frequent appearance of mucus, and in the non-occurrence of the cellular changes characteristic of oestrus. In only two of the 1625 smears examined in the present study was a characteristic oestrous picture observed. Both were taken immediately after ovariectomy.

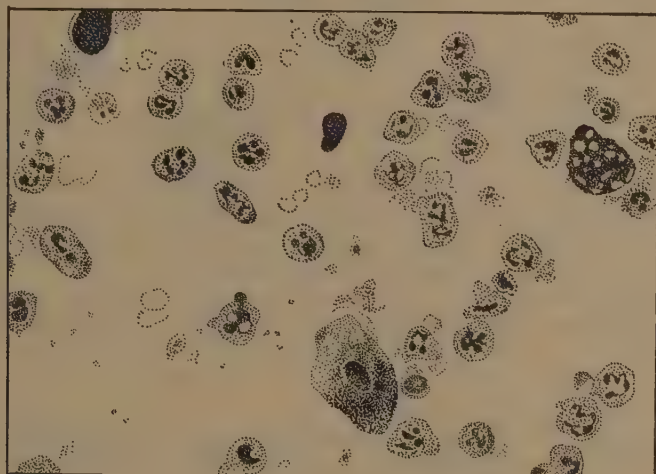


Fig. 1. Vaginal smear taken at early dioestrus. $\times 450$.

All the rats showed a regular sequence of changes in the cytological elements of the vaginal smear, similar to that reported by Kostitch & Telébakovitch (1929). The changes were, however, neither as clear nor as consistent as in a normal animal.

The smear in early dioestrus consists almost entirely of leucocytes (Fig. 1). It also contains nucleated and vacuolated basophilic epithelial cells, occasionally creased or folded acidophilic cells, large basophilic Shorr cells, and Hartman's type V 'phagocytized' cells containing one or more leucocytes surrounded by a halo. Similar cells occur in smaller numbers at all times of the cycle.

The full dioestrous smear is essentially similar to that of early dioestrus. The

concentration of leucocytes is, however, lower, the basophilic cells more vacuolated, and the number of Shorr cells and acidophilic cells reduced (Fig. 2).

Late dioestrus is not clearly defined.

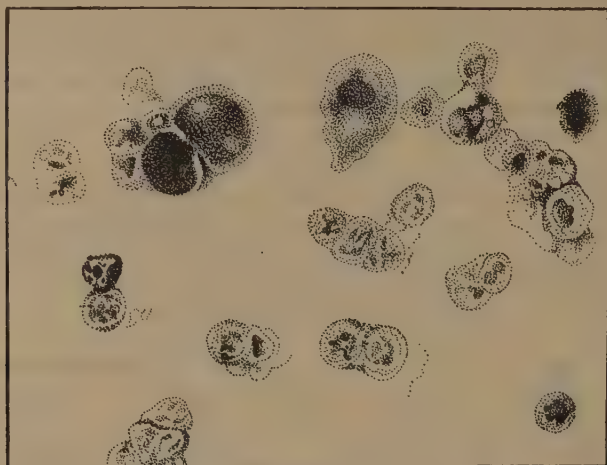


Fig. 2. Vaginal smear taken at dioestrus. $\times 450$.

The smear in 'early oestrus' consists of leucocytes, basophilic vacuolated cells, and some larger basophilic and acidophilic epithelial cells showing a clear nucleus. The latter cells, corresponding to those found in early oestrus in normal animals, appear singly or in small groups, but never in a honeycomb pattern.

The 'oestrous' smear is similar to the previous stage, but there are more acidophilic nucleated cells, corresponding in size to the fully cornified cells of the normal animal (Fig. 3).

'Late oestrus' is not clearly defined, the smear consisting of a mixture of cells found in oestrus and early dioestrus.

The duration of these six stages is discussed below.

Control rats

Control observations were made on the seven rats whose ovaries were not removed, but which were also unilaterally hysterectomized. Normal oestrous cycles occurred throughout the period of the experiment in five animals; the remaining two experienced regular oestrous cycles for only part of the period. The first animal suffered from sepsis following the operation, and the second was operated upon in very early pregnancy.

Statistical analysis

The mean duration of the cycle in the seven controls (107.4 hr.) was practically identical with that previously determined for thirty-nine unoperated rats belonging to the same colony (106.7 hr.; Mandl, 1951). The mean length of the vaginal cycle

in the nineteen spayed rats was 114 hr., and did not differ significantly from that of the controls ($P=0.7-0.6$).

The duration of the 'oestrous' phase when acidophilic cells are present was significantly shorter than the typical oestrous phase of control animals ($P=0.01-0.001$). The duration of the dioestrous phase tended to be longer, but the difference was not significant statistically ($P=0.2-0.1$). Fig. 4 shows the estimated relative lengths of the six phases.

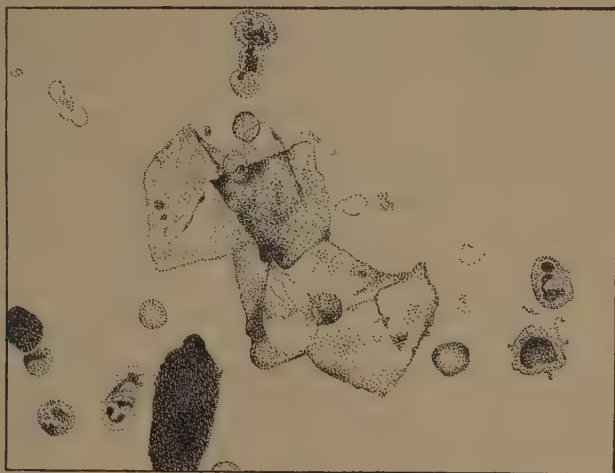


Fig. 3. Vaginal smear taken at 'oestrus'. $\times 450$.

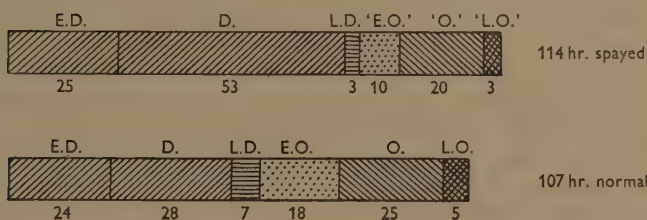


Fig. 4. The relative duration (in hours) of the six phases of the oestrous cycle in spayed and control animals. E.D.=early dioestrus; D.=dioestrus; L.D.=late dioestrus; E.O.=early oestrus; O.=oestrus; L.O.=late oestrus.

Personal error

One hundred smears were re-examined. In forty-seven there was complete agreement with the previous record; fifteen differed by $\frac{1}{2}$ stage; twenty-three by 1 stage; five by $1\frac{1}{2}$ stages and ten by 2 stages. The mean difference per reading was 0.6 stage, and was twice as great as that calculated for smears derived from normal animals (Mandl, 1951).

DISCUSSION

As already observed, macroscopic examination failed to reveal ovarian tissue in any of the animals which formed the subject of the present investigation, and all of which showed a thread-like uterus. In view of Heys's (1929) finding that some ovarian tissue regenerates (from ovarian fragments left at operation) in 7.6% of adult spayed animals, it is nevertheless conceivable that regenerated tissue was overlooked in some animals. It is highly improbable, however, that regeneration occurred in all, and consequently the present study may be regarded as fully substantiating Kostitch & Telébakovitch's (1929) view that cyclical changes take place in the vaginal smears of spayed rats. The changes occur in practically the same rhythm, but not with the same intensity, as in normal animals. Kostitch & Telébakovitch's implication that extra-ovarian oestrogen is elaborated receives support from the recent finding by Ferret (1950) that permanent oestrus may develop in spayed rats fed on a high-fat diet, and that oestrogenic substances are demonstrable in their urine. It is further supported by the finding that ovariectomy in immature animals delays the opening of the vagina but does not prevent it (Andersen & Kennedy, 1933).

The changes in the vagina of the normal animal are believed to be due to fluctuations in the level of stimulation by oestrogen, which acts directly on the vaginal epithelium. It is thus reasonable to suppose that those in the untreated spayed animal are due to variation in a 'sub-threshold' level of oestrogenic stimulation (Bourne & Zuckerman, 1940). The alternative to such a view is that they are due to some other steroid hormone. So far as we are aware, however, only oestrogen consistently stimulates proliferation of the vaginal epithelium of adult spayed animals. Other steroid hormones have different effects. For example, testosterone propionate causes vaginal opening, accompanied by a cornified smear, in immature rats, whereas its effect on the vagina of adult spayed animals is one of intense mucification (Noble, 1939). Of other steroids whose action has been reviewed by Courier (1943), only dehydroandrosterone is said to cause cornification, and then only when given in large doses to adult spayed rats; small doses cause mucification. Courier also lists other steroids, e.g. androstenediol, pregnenolone, etc., whose actions may be androgenic, progestational or oestrogenic, depending on circumstances, but he produces no experimental evidence that any of these cause vaginal cornification in adult spayed rats.

In normal animals, persistent dioestrus can be produced by testosterone (Noble, 1939; Robson, 1936), androsterone (Browman, 1937), or progesterone (Phillips, 1937); androstane-dione and androstene-dione tend to cause vaginal dioestrus, while dehydroandrosterone and *cis*-androsterone-diol cause prolonged periods of cornification (Nelson & Merckel, 1937). Spontaneously persistent oestrus (Everett, 1939) can be interrupted by means of progesterone (Everett, 1940) or by desoxycorticosterone acetate (Marvin, 1947). Such effects are, however, most probably indirect, possibly due to the inhibition of the production of gonadotrophin by the adeno-hypophysis, and the subsequent suppression of oestrogen production.

If it is assumed that the substance responsible for the vaginal cycle of spayed rats

is oestrogen, it becomes necessary to consider the probable source of oestrogens in the absence of the ovaries, and the mechanism responsible for the cyclical nature of the changes.

The most probable source is the adrenal glands (for summary of evidence, see Burrows, 1949 and Parkes, 1945). This view is consonant with Bourne & Zuckerman's (1940) observation that vaginal cycles in spayed animals maintained on a constant threshold dose of injected oestrogen occur in parallel with cyclical changes in the size of the adrenals. They have suggested that the adrenal cortex secretes a 'sub-threshold' amount of oestrogen, the level varying cyclically and reaching a maximum at oestrus. A constant threshold dose of exogenous oestrogen at which normal cyclical changes in the vaginal smears reappear supplements the small amount secreted by the animal's own adrenals, and they suggest that oestrus is the only time at which the combined supply of oestrogen is sufficiently high to evoke cornification.

The findings by Bourne & Zuckerman (1940) that cyclical adrenal changes continue after hypophysectomy and that threshold cycles could be established in spayed-hypophysectomized rats, could be taken to imply that the periodicity of the oestrous cycle in the normal rat is dependent not on the pituitary, but on the adrenals and ovaries. Experimental evidence suggests that, in adrenalectomized animals maintained on salt, oestrous cycles may continue (Kutz, McKeown & Selye, 1934), but that in spayed-adrenalectomized animals injected with a constant dose of oestrogen and extracts of the adrenal cortex, oestrous cycles cease (Bourne & Zuckerman, 1940). It may therefore be deduced that under normal conditions the ovaries play a predominant role in controlling the periodicity of changes in the accessory organs, and that after ovariectomy, these are governed by the adrenal cortex.

SUMMARY

The occurrence of cyclical changes in the vagina of spayed rats has been confirmed. The length of the cycle in nineteen spayed rats was 114 hr. as compared with 107 hr. in normal animals.

The author wishes to thank Prof. S. Zuckerman for his valuable help and advice, and Mr W. J. Pardoe for the illustrations.

REFERENCES

- ANDERSEN, D. H. & KENNEDY, H. S. (1933). The effect of gonadectomy on the adrenal, thyroid, and pituitary glands. *J. Physiol.* **79**, 1-30.
- BOURNE, G. & ZUCKERMAN, S. (1940). The influence of the adrenals on cyclical changes in the accessory reproductive organs of female rats. *J. Endocrinol.* **2**, 268-82.
- BROWMAN, L. G. (1937). Effects of androsterone and testosterone on oestrous cycle of rats. *Proc. Soc. Exp. Biol., N.Y.*, **36**, 205-8.
- BURROWS, H. (1949). *Biological Actions of Sex Hormones*, 2nd ed. Cambridge University Press.
- COURRIER, R. (1943). Action de la déhydroandrostérone sur le vagin. *Ann. Endocrinol.* **4**, 248-9.
- EVERETT, J. W. (1939). Spontaneous persistent estrus in a strain of albino rats. *Endocrinology*, **25**, 123-7.
- EVERETT, J. W. (1940). The restoration of ovulatory cycles and corpus luteum formation in persistent-estrous rats by progesterone. *Endocrinology*, **27**, 681-6.
- FERRET, P. (1950). The effect of diet on oestrogen inactivation by the liver. *Brit. J. Exp. Path.* **31**, 590-6.

- FISHER, R. A. (1946). *Statistical methods for Research Workers*, 10th ed. London: Oliver and Boyd.
- HEYS, F. (1929). Does regeneration follow complete ovariectomy in the albino rat? *Science*, **70**, 289-90.
- KOSTITCH, A. & TELÉBAKOVITCH, A. (1929). Sur un rythme vaginal chez les animaux ovariectomisés. *C.R. Soc. Biol., Paris*, **100**, 51-4.
- KUTZ, R. L., MCKEOWN, T. & SELYE, H. (1934). Effect of salt treatment on certain changes following adrenalectomy. *Proc. Soc. Exp. Biol., N.Y.*, **32**, 331-2.
- MANDL, A. M. (1951). The phases of the oestrous cycle in the adult white rat. *J. Exp. Biol.* **28**, 576-84.
- MANDL, A. M. & ZUCKERMAN, S. (1951*a*). Functional changes in the uterine vessels. *J. Endocrinol.* **7**, no. 4 (in the Press).
- MANDL, A. M. & ZUCKERMAN, S. (1951*b*). Ovarian hypertrophy after unilateral hysterectomy. *J. Endocrinol.* **7**, no. 4 (in the Press).
- MARVIN, H. N. (1947). Diestrus and the formation of corpora lutea in rats with persistent estrus, treated with desoxycorticosterone acetate. *Anat. Rec.* **98**, 383-91.
- NELSON, W. O. & MERCKEL, C. G. (1937). Effects of androgenic substances in the female rat. *Proc. Soc. Exp. Biol., N.Y.*, **36**, 823-5.
- NOBLE, R. L. (1939). Direct gynaecogenic and indirect oestrogenic action of testosterone propionate in female rats. *J. Endocrinol.* **1**, 184-200.
- PARKES, A. S. (1945). The adrenal-gonad relationship. *Physiol. Rev.* **25**, 203-54.
- PHILLIPS, W. A. (1937). The inhibition of estrous cycles in the albino rat by progesterone. *Amer. J. Physiol.* **119**, 623-6.
- ROBSON, J. M. (1936). Inhibition of oestrus and of the vaginal response to oestrone by testosterone. *Proc. Soc. Exp. Biol., N.Y.*, **35**, 49.

The Journal of
Experimental
Biology

INDEX
to
VOLS. I-15

10s. net

*To members of the Society
of Experimental Biology, 5s.*

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD
LONDON, N.W. 1

MICHROME

STAINS & REAGENTS
FOR MICROSCOPY

Chosen on merit for exhibition Festival
of Britain, Dome of Discovery

Azocarmine	Janus Green
Azur I & II	Lacmoid
Brilliant Cresyl Blue	Leishman Stain
Cedarwood Oil	May-Grunwald Stain
Cresyl Fast Violet	Nile Blue Sulphate
Field's Stain	Paraffin Wax
Giemsa Stain	Salicin, etc.

Price List on application

EDWARD GURR, LTD.

42 UPPER RICHMOND ROAD
EAST SHEEN, LONDON, S.W. 14

Telephone: Prospect 8051 & 7606
Cables: Micromlabs London

UNIFORM HIGH QUALITY, RELIABILITY,
IMMEDIATE DELIVERY

Now ready:

Microscopical Staining Techniques

No. 1 (24 pp.) 1s. 6d. No. 2 (62 pp.) 3s. 6d.

No. 3 (64 pp.) 3s. 6d.

Parasitic Animals

GEOFFREY LAPAGE

An attempt to give, from the biological point of view, an outline of the general principles which govern the lives of parasitic animals, illustrated mainly by reference to those species which attack man or the domesticated animals, with a view to showing the nature and extent of the losses inflicted by parasites on human civilization. A new volume in the Cambridge Library of Modern Science.

21s. net

CAMBRIDGE UNIVERSITY PRESS

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE

Joint Editors:

C. F. A. PANTIN, Sc.D., F.R.S. JOHN R. BAKER, D.Sc.

Third Series, No. 17

Contents of Volume 92, Part 2, June, 1951

P. C. J. BRUNET. The Formation of the Ootheca by *Periplaneta americana* I. The Microanatomy and Histology of the posterior part of the Abdomen.—E. R. TRUEMAN. The Structure, Development, and Operation of the Hinge Ligament of *Ostrea edulis*.—GORDON BLOWER. A Comparative Study of the Chilopod and Diplopod Cuticle.—J. S. ALEXANDROWICZ. Muscle Receptor Organs in the Abdomen of *Homarus vulgaris* and *Palinurus vulgaris*. With three Plates.—D. B. CARLISLE. Corpora lutea in an Ascidian, *Ciona intestinalis*.—E. J. W. BARRINGTON. The Specific Granules of the Pancreatic Islet Tissue of the Frog (*Rana temporaria*).—S. G. TOMLIN and H. G. CALLAN. Preliminary Account of an Electron Microscope Study of Chromosomes from Newt Oocytes. With one Plate (fig. 1).—M. H. HASSANEIN. Studies on the Effect of Infection with *Nosema apis* on the Physiology of the Queen Honey-bee.

Subscription Price per Volume of 4 Parts £3. 3s.

Single Parts £1. 1s. net

OXFORD UNIVERSITY PRESS LONDON E.C. 4

Journal of the MARINE BIOLOGICAL ASSOCIATION of the United Kingdom

VOLUME XXX, NO. 2 October 1951

- E. W. Knight-Jones. Gregariousness and some other aspects of the setting behaviour of *Sipirorbis*.
A. R. Hockley. On the biology of *Mytilicola intestinalis* (Steuer).
H. Barnes and S. M. Marshall. On the variability of replicate plankton samples and some applications of 'contagious' series to the statistical distribution of catches over restricted periods.
J. Bossanyi. An apparatus for the collection of plankton in the immediate vicinity of the sea-bottom.
P. G. Corbin. The seasonal abundance of young fish. XI. The year 1949.
M. Buljan. Note on a method for determination of ammonia in sea water.
Hsiao-Wei Chang. Age and growth of *Callionymus lyra* L.
Hsiao-Wei Chang. On *Callionymus reticulatus* C. & V. and its distribution in the European seas.
F. S. Russell. A re-examination of *Calanus* collected off Plymouth.
J. S. Alexandrowicz. Lymphocystis tumours in the red mullet (*Mullus surmuletus* L.).
G. R. Forster. The biology of the common prawn, *Leander serratus* Pennant.
G. R. Forster. Notes on *Leander squilla* L.
H. Barnes and T. B. Bagenal. Observations on *Nephrops norvegicus* (L.) and on an epizoic population of *Balanus crenatus* Brug.
H. Barnes and H. T. Powell. The growth-rate of juvenile *Asterias rubens* L.
C. M. Yonge. Observations on *Sphenia binghami* Turton.
Abstracts of Memoirs. Recording work done at the Plymouth Laboratory.
Marine Biological Association of the United Kingdom. Report of the Council for 1950-51.
Balance Sheet. Income and Expenditure Account.

PRICE THIRTY-THREE SHILLINGS NET

Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1

Contributors can avoid delay in the publication of their work by conforming with the following arrangements:

The Journal of Experimental Biology will publish contributions in experimental biology and comparative physiology, whether zoological or botanical. Every paper must contain a summary of the chief results of the enquiry. Authors are requested to present their work in as *concise* a form as possible. They will receive 50 copies of their papers free; additional copies, not exceeding 100 (except in special cases), may be purchased and should be ordered when the proofs are returned to the Editor.

All communications should be addressed to the Editor,

Prof. J. GRAY, Zoological Department, University of Cambridge, England

MANUSCRIPTS:

MSS. should be *typewritten* on one side only of the paper, and the pages should be numbered. All paragraphs to be printed in small type should be indicated by adding a line at the side of the copy. *Tables and Legends* should be on separate sheets from the rest of the MS. and should be numbered. The place where they are to be inserted in the text should be indicated in the margin of the MS. *Bibliography* should be given under the heading of 'References' and in the following form: Surname of authors (in alphabetical order), initials, date of publication, title of paper, title of journal (abbreviated according to the *World List of Scientific Periodicals*), volume and page of reference. In the text a reference should be quoted by the author's name and date (in brackets), and not by the numerical order of the paper in the list of references. All names of journals and books will be printed in *italics*.

Bodies of numerical data, too extensive for complete publication, should be summarized for discussion, and the originals deposited in the Archives established for this purpose at the British Museum (Natural History).

ILLUSTRATIONS:

(i) *Line blocks* should be used whenever possible. Illustrations should be pen drawings in Indian ink (*jet black and water-proof*) on smooth white Bristol board, the lettering being temporarily and lightly put in by the author with a soft pencil. It is best for the author to allow the skilled draughtsman or typesetter to deal with the final lettering. The size of the lettering inserted into the original drawing should be graded according to the degree of reduction that is desired in the final reproduction. *All brush work, tinting, or pencil shading is to be avoided*. Where considerable reduction is required, it is essential that the drawing should be of such a character as to bear such reduction.

(ii) *Plates*. Drawings should be executed on white drawing paper or card (yellowish backgrounds should be avoided), and gummed on white card grouped and numbered in the order in which they are to appear in the published plate. Exclusive of margin the plate-figures should not cover, when reduced, an area greater than $7\frac{1}{2}$ ins. in length $\times$ 5 ins. in width when ready for reproduction as a single plate or $7\frac{1}{2}$ ins. $\times$ $11\frac{1}{2}$ ins. in the case of double plates. All lettering should be put in faintly with pencil. For the reproduction of photographs by the half-tone process a good glossy bromide print is required. All lettering to appear on photographs should be shown in position on a covering sheet of transparent paper. If a part only of the photograph is required it should be indicated in the same way.

Charts and curves are frequently drawn to best advantage on *graph paper ruled in pale blue* since, whilst ensuring accuracy, the blue lines do not appear in the photograph, only the blackened lines that are desired remaining.

PROOFSHEETS:

Authors will receive one set of slip proof for correction and return to the Editor. A page proof will also be sent if the slip proof is marked by the author 'Revise'. An allowance of ten shillings per sheet of sixteen pages will be made for alterations apart from printer's errors. Authors may be charged for any excess over this average.

THE SOCIETY FOR EXPERIMENTAL BIOLOGY

The Society exists for the promotion of experimental biology. It holds Conferences, generally four times a year, at which the results of experimental investigations are presented and discussed. *The Journal of Experimental Biology* is the medium of publication of the Society. Enquiries about membership of the Society should be addressed to the Secretaries:

Dr M. M. SWANN, Department of Zoology, Cambridge

Dr W. T. WILLIAMS, Bedford College, London